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GENETICS

A PERIODICAL RECORD OF INVESTIGATIONS
BEARING ON HEREDITY AND
VARIATION

VOLUME 15 - 1930

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GENETICS

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VARIATION

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CORRIGENDA

Volume 15, 1930

- Page 423, line 5, for "II 6" read "IV 6."
Page 423, line 12, for "IV 13, IV 15" read "IV 14, IV 16."
Page 427, line 6, for "II (6)" read "II (4)."
Page 428, line 1, for "son" read "daughter."
Page 428, line 11, for "add myopia" read "and myopia."

Volume 16, 1931

- Page 8, footnote, for " $\frac{1}{e^{x^2/2\sigma^2}}$ " read " $\frac{1}{e^{x^2/2\sigma^2}}$."
Page 30, line 4 from bottom, for "55" read "45."
Page 34, line 10, for " w_i " read " w_1 "
Page 41, line 10 from bottom, for "58:" read "55:"
Page 70, figure 5, for measurement letter "E" read "F"
Page 81, line 14 from bottom, for "the production of an amount of pollen" read "the production of an amount of bad pollen."
Page 83, lines 23 and 24, for "crossing over and so forth" read "and changes in chromosome structure."
Page 93, line 16, for "sterility of a diploid" read "fertility of a diploid."
Page 108, last line of text, for "P/P'" read "P'/P'".
Page 120, last line, for " ∂v " read " ∂y ."
Page 123, line 10, for "4Nn" read "4Nu."
Page 125, line 1, for "q" read " \bar{q} ."
Page 126, line 12, for "q" read " \bar{q} ."
Page 135, line 5 from bottom, for " y^{4Ns_q} " read " e^{4Ns_q} ."
Page 141, lines 8 and 9, for "the mean gene frequencies are simply ratios of the chances of fixation at each extreme, namely, ve^{4Ns}/ve^{4Ns} " read "the mean gene frequency is simply the ratio of fixation in the favored direction to total fixation."
Page 193, line 2 from bottom, for " $1_{sII} + 6_1$ " read " $18_{II} + 6_1$."
Page 348, line 9 from bottom, for "OEHLKERS, Fr., 1921" read "R. E. O., 1921."
Page 364, line 3, for "y" read " γ ."
Page 373, line 6, for "gametic" read "zygotic."
Page 474, line 4 from bottom, for "lagging at first metaphase" read "lagging at first anaphase."
Page 491, line 11, for " e_p " read " e_v ."
Page 492, line 3 from bottom, for "or p_r " read "of p_r ."

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ERRATA, VOLUME 3

- Page 30, citation 16, following name, insert Über die Verteilung des osmotischen.
- Page 36, line 12, for $.4\text{H}_2\text{O}$ read $.4\text{H}_2\text{O}$.
- Page 41, last line, for fraction read fractionation.
- Page 62, first line of footnote, for CaCo_3 read CaCO_3 .
- Page 79, text lines 16 and 18, for xanthoproteic read xanthoproteic.
- Page 113, line 7, for homogeneity read homogeneity.
- Page 169, line 5, for heighth read height.
- Page 174, line 13, for flash read flask.
- Page 183, line 8, for landwirtschaftlichen read landwirtschaftlichen.
- Front cover, July no., title 5, for hardness read hardness.
- Page 239, control solution, for $\text{Al}_2(\text{SO}_4)_3$ and CuSO_4 , read $\text{Al}_2(\text{SO}_4)_3$ and CuSO_4 .
- Page 309, text line 7, for findinge read findings.



Nägeli

FURTHER GENETICAL STUDIES OF *APLOCHEILUS LATIPES*

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Received February 25, 1929

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INTRODUCTION

The genetic studies on a fresh-water fish, *Aplocheilus latipes*, carried on up to date since 1921, when my earlier investigations were reported, have revealed some new and interesting facts. The present paper is a report upon them. As it is a continuation of the former paper, I will here summarize some of the former results which are necessary to an understanding of the present experiments.

Among the color varieties of *Aplocheilus latipes*, orange red (briefly red) and white are sex-linked. Red (*R*) is dominant to white (*r*) and both genes are located in the sex chromosomes, X or Y. The sex inheritance is of the *Drosophila* type, the female being homogametic and the male heterogametic. The homozygous red male possesses the dominant gene *R* in both the X and the Y chromosomes. The cross of white female by red male produces heterozygous red offspring of both sexes, females **XXR_r** and males **X_rYR**. When the heterozygous red male **X_rYR** is mated to white females **X_rX_r**, all male offspring are red and the females white. Here the sex linkage of red and white is well manifested. As the epigenetic gene *R* is located in the Y chromosome, it is inherited only in the masculine line, and the male is always red and the female white; and this strain may be perpetuated by mating the offspring *inter se*. Sometimes crossing over between X and Y chromosomes takes place, and the gene *R* is transferred from Y to X and there result red females (**XXR_r**) and white males (**X_rY_r**).

A white male **X_rY_r** mated with a heterozygous red female **XXR_r** yields red and white offspring in both sexes and in equal proportions (**XXR_r**,

\mathbf{XrXr} , \mathbf{XRYr} , \mathbf{XrYr}). Among these offspring the red male possesses the R gene not in a Y but in an X chromosome, in contrast to the original red male.

To determine the frequencies of the transfer of the R gene from the X to the Y chromosome, and conversely, from the Y to the X , was the principal aim of the present experiments. For this purpose the two kinds of heterozygotic red male, \mathbf{XrYR} and \mathbf{XRYr} , were repeatedly crossed with white females, \mathbf{XrXr} , and the exceptional offspring which resulted from crossing over were counted. If the two chromosomes, X and Y , were homologous, and crossing over took place between them with regularity, the frequency of transfer of the R gene from Y to X and from X to Y would be equal. A remarkable difference, however, was found between them. Moreover, a striking exceptional red male was found among the offspring of an \mathbf{XRYr} male, and breeding tests showed that it must have the genetical formula \mathbf{XXY} .

CROSSING OVER BETWEEN THE X AND THE Y CHROMOSOMES

(a) *Transfer of the R gene from Y to X .*

Heterozygous red males (\mathbf{XrYR}) were repeatedly mass-crossed to white females (\mathbf{XrXr}) and the production was noted of exceptional red females (\mathbf{XRXr}) and white males (\mathbf{XrYr}). These exceptional forms result from crossing over,—in this case, transfer of the R gene from the Y to the X chromosome. Their numbers are given in table 1.

TABLE 1
Red males (\mathbf{XrYR}) \times White females (\mathbf{XrXr}).

YEAR OF BREEDING	NORMAL OFFSPRING		EXCEPTIONAL OFFSPRING	
	WHITE FEMALE, \mathbf{XrXr}	RED MALE, \mathbf{XrYr}	RED FEMALE, \mathbf{XRXr}	WHITE MALE, \mathbf{XrYr}
1919	156	148	3	0
1920	147	154	0	3
1922	754	748	1	8
1923	349	373	0	0
1924	724	708	1	1
1925	1817	1945	4	7
	3947	4076	9	19
	8023		28	

The ratio of the exceptional to normal offspring is about 1:300

(b) *Transfer of the R gene from X to Y .*

The heterozygous red males (\mathbf{XRYr}) which have the R gene in an X

chromosome, and which are the offspring of a heterozygous red female mated to a white male, were mass-crossed to white females $XrXr$. The normal offspring were red females ($XXRr$) and white males ($XrYr$), but some exceptional white females and red males were obtained. The results are summarized in table 2.

TABLE 2
Red male ($XXRYr$) \times White female ($XrXr$).

YEAR OF BREEDING	NORMAL OFFSPRING		EXCEPTIONAL OFFSPRING	
	RED FEMALE, $XrXr$	WHITE MALE, $XrYr$	WHITE FEMALE	RED MALE
1921	484	450	2	0
1922	511	465	0	2 (A, B)
1923	574	574	2	4 (C, D, E, F)
1924	994	975	1	3 (G, H, J)
	2563	2437	5	9
	5000		14	

The ratio of the exceptional to normal offspring is about 1:360. It is very near to that in table 1, so that, at first sight, one might be induced to conceive all of these exceptional forms as the result of crossing over between X and Y chromosomes. The breeding experiments, however, showed that two males, A and B, from the 1922 breeding, and two males, C and D, from the 1923 breeding, and all three males, G, H, and J from the 1924 breeding had an unexpected genetic constitution resulting from non-disjunction of X, and being in formula $XXRrYr$, and not $XrYR$, as expected, as will be shown later in this paper.

The two remaining males, E and F, were true crossover forms and when mated to white females produced white females and red males in equal proportions. The offspring of E were 22 white females and 22 red males; the offspring of F were 83 white females and 86 red males.

As to the exceptional white females, I was unable to obtain an appropriate mate to determine whether they had resulted from crossing over, and so had the genetic formula $XrXr$, or from non-disjunction, and so had the genetic formula XrO . Consequently, in calculating the frequency of crossing over, it will be safe to neglect the exceptional females and to take into account the males only.

The total sum of the males is 2446, of which two (E, F) are crossovers, the ratio being about 1200:1. The difference between this ratio and that mentioned in table 1 (1:360) is too great to be passed by with-

out any consideration. The *R* gene was found to have removed from the Y chromosome to the X about four times oftener than from X to Y, or, in other words, the Y chromosome has lost the *R* gene much easier than has the X. A similar difference was found by WINGE (1923). A gene, *elongatus* (e_1), which produces the elongation of the caudal fin in *Lebistes*, removed from the Y chromosome to the X four times out of 68 and from the X chromosome to the Y only once in 73 cases. Nevertheless, WINGE (1923, 1927) has concluded that the X and Y chromosomes are essentially homologous, and that the only difference between them consists in the location of an epistatic male-determining gene in the Y chromosome. He did not lay much stress upon this difference, though he indicated it as remarkable.

If the crossing over between the X and Y chromosomes takes place simply by chance and no other cause interferes with it, the frequencies of the transfer of the *R* gene in both directions should be equal, and no preponderance on either side should take place. At present I am unable to elucidate the cause of this difference in the direction of crossing over. Whether it is effected by a dissimilarity in structure of the X and Y chromosomes or by the action of some gene or genes situated in one of them is not known. However, the tendency of the Y chromosome more frequently to lose the dominant gene and less often to regain it is well established in our fish and also in *Lebistes*; and if this difference may be supposed to occur also in other animals, we have here, perhaps, a plausible explanation of the origin of the so-called empty Y chromosome of *Drosophila* where crossing over must have taken place so repeatedly between the X and Y chromosomes that the Y chromosome has gradually lost its dominant genes, eventually to become quite empty.

NON-DISJUNCTIONAL MALES

The exceptional red males A, B, C, D, G, H, and J of table 2, which showed no peculiarity in structure, either macroscopic or microscopic, of their genital glands, when crossed with the white females, produced the remarkable results shown in table 3. The progeny were mainly females, red and white in equal proportions, while the males were few in number or none at all.

The total results are 996 red and 953 white females and 11 red and 8 white males. Were the genetic constitution of the exceptional males $XrYR$, which is to be expected if they were produced by the crossing over of the *R* gene from the X to the Y chromosome, their progeny should be white females and red males in equal numbers. As the experimental re-

TABLE 3

Primary non-disjunctional red male × white females (in all crosses 3 females were mated to a single male).

NUMBER AND YEAR OF BREEDING	MALE PARENT	OFFSPRING				RATIO
		RED ♀	WHITE ♀	RED ♂	WHITE ♂	
3 B. 1923	A	33	32	0	0	30:1
4 B. 1923	B	55	36	2	1	

(These three male offspring, two red and one white, were lost by an unfortunate accident before they were genetically studied).

3 B. 1924	C	20	26	0	1 (K)	50:1
2 B. 1925	C	265	271	0	0	
4 B. 1924	D	9	25	0	0	250:1
3 B. 1925	D	255	260	1 (L)	1 (M)	
4 B. 1925	G	78	64	0	0	200:1
5 B. 1925	H	125	91	1 (N)	0	
6 B. 1925	J	156	148	7 (P ₁ - P ₇)	5 (P ₈ - P ₁₂)	25:1
Total		♀ 1949		♂ 19		100:1

sults do not correspond to this expectation, they are evidently not the product of crossing over, and their genetic constitution should be different from this expectation. The production of the red and white females makes it necessary to consider that the male parent had two X's, an \mathbf{XR} and an \mathbf{Xr} . The genetic formula having a double dose of X is characteristic of females, but as these individuals are male, not female, they must necessarily have some male-determining factor which is more powerful than two X's. The phenomenon may be explained fairly well by the supposition that all these exceptional red males are the result of non-disjunction between sex chromosomes and have a Y chromosome in addition to two X's. The presence of a Y chromosome in these males will be fully substantiated when they, or their male progeny, produce the normal male of the constitution XY, which yields males and females in equal proportions. Such males were obtained, actually, among their offspring. Such, for example, were the red male N derived from male H, reared in breeding 5 in 1925 (table 3), and the white male K_{2,1}, a great-grandson of male C, reared in breeding 2 in 1926. (See table 4).

WINGE (previously cited), investigating crossing over between the X and the Y chromosomes in *Lebistes*, concluded that the Y chromosome carries a dominant male-determining gene. His conclusion is based on the supposed homology between the X and the Y chromosomes, inferred from

TABLE 4, PART 1

Offspring of secondary non-disjunctional males (In all crosses 3 females were mated to a single male).

NUMBER AND YEAR OF BREEDING	PARENTS		OFFSPRING				TOTAL		RATIO
	♂	♀	♀	♀	♂	♂	♀	♂	
4 B. 1925	K white (3B. 1924)	Sister white		129 white		10 (K ₁ — K ₁₀) white	129	10	10:1
11 B. 1926	L red (3B. 1925)	White	461 red	383 white	0	0	844	0	
12 B. 1926	M white (3B. 1925)	Wild brown	232 brown		1 (M ₁) brown		232	1	200:1
13 B. 1926	M white (3B. 1925)	Sister red	113 red	108 white	1 (M ₂) red		221	1	
14 B. 1926	N red (5B. 1925)	Wild brown	133 brown		156 brown		133	156	1:1
15 B. 1926	P ₁ red (6B. 1925)	Red	190 red	68 white	0	0	258	0	
16 B. 1926	P ₂ red (6B. 1925)	White	281 red	268 white	0	1 (P _{2,1}) white	549	1	500:1
17 B. 1926	P ₃ red (6B. 1925)	Red	237 red	83 white	3 (P _{3,1} — P _{3,3})* red	1 (P _{3,4}) white	320	4	80:1
18 B. 1926	P ₄ red (6B. 1925)	Wild brown	248 brown		0		248	0	
19 B. 1926	P ₅ red (6B. 1925)	Wild brown	303 brown		0		303	0	
20 B. 1926	P ₆ red (6B. 1925)	White	130 red	138 white	1 (P _{6,1}) red	4 (P _{6,2} — P _{6,5}) white	268	5	50:1
21 B. 1926	P ₇ red (6B. 1925)	White	246 red	238 white	0	0	484	0	
22 B. 1926	P ₈ white (6B. 1925)	White		257 white		1 (P _{8,1}) white	257	1	250:1
23 B. 1926	P ₉ white (6B. 1925)	Red	250 red	240 white	0	1 (P _{9,1}) white	490	1	500:1
24 B. 1926	P ₁₀ white (6B. 1925)	Wild brown	343 brown		0		343	0	
25 B. 1926	P ₁₁ white (6B. 1925)	White		380 white		1 (P _{11,1}) white	380	1	400:1
26 B. 1926	P ₁₂ white (6B. 1925)	Red	98 red	86 white	0	0	184	0	
Total, exclusive of the offspring of N							5510	25	

* P_{3,2} was an abnormal male and yielded no offspring.

TABLE 4, PART 2

NUMBER AND YEAR OF BREEDING	PARENTS		OFFSPRING				TOTAL		RATIO
	♂	♀	♀	♀	♂	♂	♀	♂	
1 B. 1926	K ₁ white (4B. 1925)	Red	189 red	173 white	0	1 (K _{1,1}) white	362	1	350:1
2 B. 1926	K ₂ white (4B. 1925)	Sister white		264 white		4 (K _{2,1} — K _{2,4}) white	264	4	70:1
3 B. 1926	K ₃ white (4B. 1925)	Sister white		200 white		4 (K _{3,1} — K _{3,4})white	200	4	50:1
4 B. 1926	K ₄ white (4B. 1925)	Wild brown	346 brown		0		346	0	
5 B. 1926	K ₅ white (4B. 1925)	White		280 white		0	280	0	
6 B. 1926	K ₆ white (4B. 1925)	Wh		346 white		4 (K _{6,1} — K _{6,4})white	346	4	90:1
7 B. 1926	K ₇ white (4B. 1925)	Red	117 red	124 white	1 (K _{7,1}) red	0	241	1	240:1
8 B. 1926	K ₈ white (4B. 1925)	White		142 white		0	142	0	
9 B. 1926	K ₉ white (4B. 1925)	Red	177 red	189 white	0	1 (K _{9,1}) white	366	1	360:1
10 B. 1926	K ₁₀ white (4B. 1925)	White		360 white		8 (K _{10,1} — K _{10,8}) white	360	8	40:1
24 B. 1927	M ₁ brown (12B. 1926)	White	59 brown 38 red	50 blue 24 white	0	0	171	0	
25 B. 1927	M ₂ red (13B. 1926)	Red	127 red	58 white	13 red	6 white	185	19	10:1
26 B. 1927	P _{2,1} white (16B. 1926)	White variegated		55 white variegated 40 white		0	95	0	

TABLE 4, PART 2 (continued)

NUMBER AND YEAR OF BREEDING	PARENTS		OFFSPRING				TOTAL		RATIO
	♂	♀	♀	♀	♂	♂	♀	♂	
27 B. 1927	P _{3,1} red (17 B. 1926)	White	24 red	26 white	0	0	50	0	
28 B. 1927	P _{3,3} red (17B. 1926)	White	25 red	15 white	0	0	40	0	
29 B. 1927	P _{3,4} white (17B. 1926)	Red	87 red	68 white	2 red	1 white	155	3	50:1
30 B. 1927	P _{6,1} red (20B. 1926)	White	74 red	53 white	0	0	127	0	
31 B. 1927	P _{6,2} white (20B. 1926)	Red	22 red	23 white	1 red	0	45	1	50:1
32 B. 1927	P _{6,3} white (20B. 1926)	Red	61 red	42 white	0	1 white	103	1	100:1
33 B. 1927	P _{6,4} white (20B. 1926)	Red	47 red	47 white	4 red	1 white	94	5	20:1
34 B. 1927	P _{6,6} white (20B. 1926)	Red	58 red	60 white	2 red	7 white	118	9	10:1
35 B. 1927	P _{8,1} white (22B. 1926)	Red	49 red	31 white	0	0	80	0	
36 B. 1927	P _{9,1} white (23B. 1926)	Red	65 red	69 white	8 red	11 white	134	19	7:1
37 B. 1927	P _{11,1} white (25B. 1926)	Brown	49 brown 22 red	33 blue 23 white	0	1 blue	127	1	120:1

Total 4431 81

TABLE 4, PART 3

NUMBER AND YEAR OF BREEDING	PARENTS		OFFSPRING				TOTAL		RATIO
	♂	♀	♀	♀	♂	♂	♀	♂	
1 B. 1927	K _{1,1} white (1B. 1926)	Sister red	81 red	78 white	1 red	1 white	159	2	80:1
2 B. 1927	K _{2,1} white (2B. 1926)	White variegated		75 white variegated 31 white		79 white variegated 32 white	106	111	1:1
3 B. 1927	K _{2,2} white (2B. 1926)	White variegated		34 white variegated 23 white		17 white variegated 10 white	57	27	2:1
4 B. 1927	K _{2,3} white (2B. 1926)	Brown	71 brown 50 red	51 blue 52 white	2 brown	1 blue	224	3	70:1
5 B. 1927	K _{2,4} white (2B. 1926)	Red	90 red	66 white	17 red	11 white	156	28	6:1
6 B. 1927	K _{2,1} white (3B. 1926)	Brown	30 brown 38 red	31 blue 33 white	2 red	1 white	132	3	40:1
7 B. 1927	K _{2,2} white (3B. 1926)	Brown	52 brown 55 red	37 blue 31 white	3 red	1 blue	175	4	40:1
8 B. 1927	K _{2,3} white (3B. 1926)	Brown	27 brown 13 red	24 blue 16 white	0	0	80	0	
9 B. 1927	K _{2,4} white (3B. 1926)	Brown	20 brown 14 red	18 blue 6 white	0	1 blue	58	1	60:1
10 B. 1927	K _{2,1} white (6B. 1926)	White variegated		2 white variegated 2 white		0	4	0	
11 B. 1927	K _{2,2} white (6B. 1926)	Red	75 red	76 white	10 red	4 white	151	14	10:1
12 B. 1927	K _{2,3} white (6B. 1926)	Red	70 red	59 white	4 red	1 white	129	5	30:1
13 B. 1927	K _{2,4} white (6B. 1926)	Red	47 red	47 white	7 red	7 white	94	14	7:1
14 B. 1927	K _{7,1} red (7B. 1926)	White	49 red	34 white	4 red	8 white	83	12	7:1

TABLE 4, PART 3 (continued)

NUMBER AND YEAR OF BREEDING	PARENTS		OFFSPRING				TOTAL		RATIO
	♂	♀	♀	♀	♂	♂	♀	♂	
15 B. 1927	K _{9,1} white (9B. 1926)	Red	71 red	72 white	5 red	13 white	143	18	8:1
16 B. 1927	K _{10,1} white (10B. 1926)	Brown	61 brown 29 red	64 blue 21 white	1 brown	0	175	1	170:1
17 B. 1927	K _{10,2} white (10B. 1926)	Brown	91 brown 47 red	65 blue 40 white	1 brown 1 red	2 blue 1 white	243	5	50:1
18 B. 1927	K _{10,3} white (10 B. 1926)	Brown	90 brown 68 red	86 blue 57 white	2 brown 2 red	1 blue 2 white	301	7	40:1
19 B. 1927	K _{10,4} white (10 B. 1926)	Brown	60 brown 44 red	53 blue 25 white	2 red	2 blue 1 white	182	5	40:1
20 B. 1927	K _{10,5} white (10B. 1926)	Brown	64 brown 38 red	51 blue 39 white	0	1 white	192	1	200:1
21 B. 1927	K _{10,6} white (10B. 1926)	Red	139 red	141 white	4 red	2 white	280	6	50:1
22 B. 1927	K _{10,7} white (10B. 1926)	Red	139 red	131 white	7 red	6 white	270	13	20:1
23 B. 1927	K _{10,8} white (10B. 1926)	Red	79 red	72 white	23 red	20 white	151	43	4:1

Total, exclusive of the offspring of K_{2,1} and K_{2,2}

3382 185

the occurrence of crossing over between them. But, as already described, the crossing over in our fish and also in *Lebistes* does not occur with equal frequency in both directions so that the homology of an X to a Y chromosome is questionable. The existence of a dominant male-determining gene or genes in the Y chromosome, however, is fully established

in the present case, where the non-disjunctional forms XXY are found to be males.

All the seven exceptional red males above mentioned were primarily non-disjunctional. They were products of the mass-breeding of heterozygous red males (XYr) with white females ($XrXr$). A question may be raised as to whether some of their parents might not have been previously non-disjunctional, and they themselves secondary forms. But when we examine the pedigree of the parents there will be no room for doubt. We will consider the male side first. If some of the parental red males were non-disjunctional, their mating to white females should have produced white females in abundance, just as happened in the mating of the primary non-disjunctional males of table 3. But this did not happen here, for the white females were very few, even less than the non-disjunctional males (table 2). Second, the possibility that some white female parents might have been non-disjunctional and of the formula $XrXrXr$ is also negligible when the genetic constitution of their progenitors is investigated. They were the progeny of heterozygous red females $RRXr$ and white males $XrYr$, which produced red and white females and males in equal proportions. Any non-disjunction among $RRXr$ and $XrYr$ is unable to produce $XrXrXr$ forms.

Now, inasmuch as these seven non-disjunctional males were red, it is clear that the non-disjunction took place in the male parent. Consequently, an XR and a Yr chromosome failed to be disjoined in the reduction division, and XYr sperms were produced. The XYr sperms fertilizing Xr ova produced $RRXrYr$ zygotes which developed into non-disjunctional males.

Considering those exceptional red males as the primary non-disjunctional individuals, we are now in a position to calculate their frequency. As the exceptional white females were not tested, as already mentioned, the red individuals only are to be taken into account. The total of the red females is 2563 and to them the non-disjunctional red males are as 1:360. In *Drosophila melanogaster* the total of the results of the experiments on primary non-disjunction obtained by BRIDGES, SAFIR, and MAVOR gives the ratio 1:2000 (MORGAN 1925), and the exceptional highest ratio among the results of SAFIR's experiments (SAFIR 1920) on five different stocks is 1:550. The ratio in our fish is about six times higher than the normal ratio in *Drosophila* and approaches near to the exceptional high ratio of SAFIR. Though, inasmuch as in *Drosophila* the non-disjunction takes place in females, while in our fish it occurs in males, it will not be strictly rational to compare the two ratios, it is, how-

ever, apparent that in *Aplocheilus* the primary non-disjunction takes place much more frequently than in *Drosophila*.

SECONDARY NON-DISJUNCTIONAL MALES

The seven primary non-disjunctional red males mated to white females produced 1949 female offspring (some red, some white) and 19 male offspring (red and white, table 3). Of the 19 male offspring 3 were lost so that only the remaining 16 were genetically tested. They were all, except one, the red N, secondary non-disjunctional, and when mated to females of any kind—sisters, the wild form, or other color varieties—produced, like their male parents, mainly female offspring, but usually with a few male offspring. The latter were, with rare exceptions, also non-disjunctional. The results of the breeding tests of these males, generation after generation, are gathered in table 4.

The offspring of 16 sons (K, L, M, N, and $P_1 \cdots P_{12}$) of the primary non-disjunctional males are listed in the first part of the table. Among them the single one N produced, with wild brown females, 133 female and 156 male offspring (14B. 1926). This sex ratio is very close to the normal ratio 1:1 and the result of the breeding of offspring *inter se* in the next year showed also a normal sex ratio, so that N may safely be considered a normal male of the sexual constitution XY. This appearance of a normal male offspring is a convincing proof that the Y chromosome was present in their progenitor, the H male, and that the latter possessed Y in addition to two X chromosomes, as already mentioned.

The remaining 15 males were all secondary non-disjunctional of the first generation and produced in total 5535 offspring, of which 25 were male, the ratio being 200:1. The ratio in each culture varies between 0 and 10:1.

One male ($P_{3,2}$) of the 25 male offspring was an abnormal form unable to mate and yielded no offspring. The other 24 were all non-disjunctional and produced in total 4431 females and 81 males in the ratio of 55:1 (table 4, part 2). The production of males was about 3.5 times larger than in the first generation. The range of variation of the ratio in different cultures was naturally wider, extending from 0 to 7:1.

In 1927 I was able to rear offspring of the third generation of K-lineage (table 4, part III). Among 23 male offspring ($K_{1,1}, K_{2,1} \cdots K_{2,4}, K_{3,1} \cdots K_{3,4}, K_{6,1} \cdots K_{6,4}, K_{7,1}, K_{9,1}, K_{10,1} \cdots K_{10,8}$) of the secondary nondisjunctional males of the second generation, one male ($K_{3,1}$) was normal and yielded 106 female and 111 male offspring (2B. 1927). Another male ($K_{2,2}$) produced 57 female and 27 male offspring in the ratio 2:1

(3B. 1927). The number of the male offspring is here too great and the ratio approaches near to the most deviating sex ratio (3:2) ever found by me among my many years' breeding experiments on the normal individuals. So it will not be safe to consider $K_{2,2}$ as non-disjunctional, but the positive determination by its male offspring must be awaited until the next summer. At present, it is neglected in counting. Leaving aside these two individuals, the 21 other males produced a total of 3382 females and 185 males, the ratio being 20:1. The male ratio is here about thrice as large as that in the second generation and tenfold that in the first generation. The ratio in the different cultures varies between 0 and 4:1.

From all above stated, we see that the production of males in the offspring of non-disjunctional males increases exceedingly from generation to generation. What caused this increase — whether the presence of some gene or some local external condition—is not known at present. I hope that further investigation, now undertaken, will in the future throw some light upon this point.

The offspring of all non-disjunctional males, primary and secondary, consists, in total, of 15,272 females and 310 males. Among the males 63 were genetically tested, and only two were found to be normal, and the remaining 61 were all non-disjunctional. From these facts we may conclude that the non-disjunctional males of *Aplocheilus latipes*, either primary or secondary, produce chiefly female offspring and few non-disjunctional males, so that the lineage of non-disjunction may be perpetuated simply by breeding the male offspring with any normal female from generation to generation. The production of normal males was very rare; 2 out of 65 males were normal.

Since hitherto I have been unable to engage in cytological investigation, no evidence has yet been obtained about the behavior of the non-disjunctional chromosomes. It is however highly probable that in the reduction division of the non-disjunctional male the homosynapsis of two X chromosomes normally takes place among the three chromosomes X, X, and Y, and that at the stage of the separation of chromosomes to two poles the superfluous solitary Y chromosome lagging in the middle of the spindle might be excluded from the formation of the gametic nuclei. Owing to such a process, all gametes of the non-disjunctional male parent might have one X chromosome and consequently only females be produced. In some cases, the Y chromosome may enter with the X chromosome into one of the nuclei and form a gamete possessing X and Y chromosomes, which will produce secondary non-disjunctional males. The heterosynapsis XY may also occur but very rarely. In this case, one X is the lagging

chromosome and is generally eliminated, and consequently two gametes are produced, one having the X and the other the Y chromosome. The gamete possessing Y will produce the normal male offspring. The total number of the offspring of the non-disjunctional males whose male offspring were genetically tested was 10,274 (the sum of 7 cultures of primary non-disjunctional males and the cultures of secondary non-disjunctional males in 1925 and 1926), out of which only two were normal males. But the heterosynapsis will not be the sole way of the production of normal male. The elimination of X in the course of the development of XXY ovum will also produce a normal male. The red N male is the offspring of the red non-disjunctional male $XXR \cdot Yr$ mated to white female $Xr \cdot Xr$. Heterosynapsis between XR , Xr and Yr chromosomes is unable to produce R carrying sperm which will produce, with an X ovum, a normal male unless R crosses over from X to Y chromosome. It is more reasonable to conjecture the formation of an N individual by elimination of one Xr in $XXR \cdot Yr$ ovum than to suppose a concurrent occurrence of crossing over and rare heterosynapsis. If the synapses in an XXY male takes place at random, the number of XY synapses should be twice as great as that of XX synapses and one third of the whole number of gametes would receive the disjoined Y chromosome and produce a normal male, except in a few cases where it is accompanied by the lagging chromosome. The scarcity of normal males shows that there must be a strong preference for homosynapsis. It is exceptionally strong in our fish and much stronger than in *Drosophila melanogaster*, where BRIDGES (previously cited) has observed homosynapsis 83.5: heterosynapsis 16.5. In *Aplocheilus*, indeed, the frequency of the occurrence of heterosynapsis in non-disjunctional males may be well conceived roughly to be zero.

This strong preference for homosynapsis with the preponderance of one-sided crossing over, as already mentioned, seems to indicate some morphological or physiological difference between the X and Y chromosomes in our fish, and does not support the supposition of WINGE that X and Y chromosomes in *Lebistes* are homologous, differing only in the sex-determining genes contained.

NEW MUTANTS, "FUSED" AND "WAVY"

Some aberrant types of the vertebræ appeared from time to time in our stocks. Sometimes the vertebral bodies ankylose together, and when the ankylosis takes place in many vertebræ, the vertebral column is much shortened, and the fish shows a dwarf form. In other cases, the vertebral column curves wavily, dorso-ventrally, and shows in side view

a high or low undulation. It also curves in some fishes wavily laterally, or sometimes bends the caudal portion simply to the left or right side. Occasionally, only the terminal vertebra bends laterally and makes a small prominence at the base of the caudal fin. Breeding tests show that the two last mentioned aberrations are not inherited and may be looked upon as developmental abnormalities. The former two, "fused" and "wavy," are heritable mutants, and some description of them will here be given.

"Fused." This is a character recessive to the normal and independent of all known color genes and of sex. It varies in its intensity somewhat widely, and, in an extreme case, nearly all the vertebræ fuse into a few pieces, so that the fish shows a strong dwarfishness. Such a form may easily be distinguished externally from the normal even in an early stage of its growth. When only a few vertebræ fuse, the character is impossible to detect externally, and can be recognized only after examination by dissection.

This mutant is much less viable than the normal. Especially when it is cultured together with the normal, its death rate is greatly increased. Hence, in counting it in a culture, I first selected the individuals which were conspicuously dwarf and in which the fusion takes place a few weeks after hatching. These were included in a preliminary count. The remaining fishes were cultured till they grew nearly to their full size, and their vertebrae were examined after dissection. The number of the mutants then obtained was added to the first count, and the final results were thus obtained. As the death of some individuals is inevitable in a culture which is continued about two months in order to let the fish reach their full size, naturally the number of the mutants obtained is much less than the expectation.

The F_1 offspring of fused mated to the normal were normal, and of the F_2 , 3212 were normal and 988 fused. In a back cross with fused, the heterozygous F_1 produced 837 normal and 750 fused.

"Wavy." The vertebral column in this mutant curves dorso-ventrally and forms generally two wavy crests. The external body form shows a more or less wavy outline dorsally as well as ventrally, according to the undulation of the vertebral column. This character is also recessive to the normal and independent of all other known genetic characters and of sex. It was found more than once in our stocks, especially in the heterozygous fused stock, but the breeding tests showed no linkage between it and "fused."

The F_1 offspring of "wavy" mated to normal were all normal and F_2

was composed of 1528 normals and 469 wavies. In back cross with "wavy," the F_1 yielded 643 normals and 503 wavies. These results do not fit strictly the expected proportions of 3:1 and 1:1, but as they were cultured in mass, the weak "wavy" together with the strong normal, differential viability might be expected, and so, a deficiency of "wavy" individuals.

In pure matings of these two mutants, a few individuals have reverted to the normal form. One out of 70 fused, and one out of 80 wavies were in form normal, and both were heterozygous.

In other species of fish, I have observed rarely abnormal forms similar to the above mentioned. The fused type was found in *Pagrus tumifrons* T. and S., *Cyprinus carpio* L., and *Carassius auratus* (L.); and the wavy type in *Cyprinus carpio* L. and *Plecoglossus altivelis* T. and S. These abnormalities seem to be widely spread among fishes.

SUMMARY

The inequality of the frequencies of transfer of a dominant gene from the Y to the X chromosome and from the X to the Y chromosome is described. The *R* gene, which is responsible for the formation of yellow pigment, crosses over from Y to X with a frequency of 1:300, while it returns from X to Y with a frequency of 1:1200. There is a large preponderance of the crossing over from Y to X rather than from X to Y.

The non-disjunctional form XXY in *Aplocheilichthys latipes* is male. It produces with any normal female mainly female offspring, with a few non-disjunctional males, or none.

The frequency of the occurrence of primary non-disjunction is 1:360. The frequency of secondary non-disjunction increases with each successive generation; at the third generation it was tenfold that of the first generation.

Two recessive mutants, "fused" and "wavy," are described.

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AN EXPERIMENTAL STUDY OF HYBRID VIGOR OR HETEROSIS IN RATS¹

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INTRODUCTION

A review of the literature of both plant and animal hybridization shows that increased vigor of growth and reproduction usually accompanies hybridization although it is by no means a universal phenomenon. The term hybridization is here used as it is commonly employed by students of genetics rather than in the restricted sense sometimes ascribed to it in systematics. It applies to any cross between individuals of different genetic constitution, whether or not they belong to different Linnean species, or merely to different varieties, breeds or families of the same species.

HYPOTHESES ADVANCED AS AN EXPLANATION OF HYBRID VIGOR

The fact that the early plant and animal hybridizers did not know of any mechanism of heredity accounts for their failure to attempt to give any explanation as to the cause of hybrid vigor. Animal breeders, however,

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did make use of both inbreeding and crossbreeding in the early development of many breeds of farm animals. They realized that while close-breeding tended to standardize type, such a system of breeding often concentrated undesirable characters, such as loss of vigor and size, low fertility and susceptibility to disease. Their antidote for these bad conditions was the use of an unrelated sire, and as a rule more vigorous and productive offspring were the result of such an outcross.

DARWIN (1877) could not see why the bad qualities of inbred plants and animals could be caused by a concentration of such qualities, because he knew of many cases of inbred plants and animals of different strains or coming from different herds but possessing the same or similar undesirable qualities, which when crossed, gave vigorous progeny. Cases of this kind proved to him the great advantage of crossing even though the individuals crossed were weak or carried undesirable qualities. He attributed the hybrid vigor to the bringing together of diverse sexual elements from compatible but somewhat unrelated types, and not to the mere act of crossing. His closing arguments on the value of cross-fertilization are based on the fact that there must be a difference in the sexual elements before hybrid vigor is possible. DARWIN believed that the environment of individual plants or animals was directly associated with the change in, or the differentiation of the sexual elements. He seems to have been the first to offer an explanation of hybrid vigor.

DAVENPORT (1908) spoke of the influence of dominant and recessive factors in a pair of Mendelian allelomorphs. He believed the dominant factor to be the progressive one while the recessive was retrogressive and often lacked in vigor. From this point on, two general hypotheses of hybrid vigor have been advanced.

In brief, one of these hypotheses is based upon the degree of heterozygosity brought about by crossbreeding, that is, the more nearly crossbreeding brings about a 100 percent heterozygous condition of all allelomorphic pairs of genes the more vigorous the hybrid will be. This conception of hybrid vigor led to the acceptance of "heterosis" (G. H. SHULL) as a logical word to express the increased growth or vigor possessed by many hybrid animals and plants. The second of these hypotheses is based upon the number of dominant genes which are brought together in hybrids and the greater the number of dominants, it is supposed, the more clearly will hybrid vigor be manifested. A modification of the second hypothesis regards certain dominant genes as contributing more largely than others to the vigor of the offspring, or even as having a predominant influence on vigor so that they can be regarded as specific "growth genes."

Three investigators seem to have been interested in the dominant hypothesis as an explanation of hybrid vigor at about the same time. BRUCE (1910) points out that the total number of dominant factors is greater in a hybrid population than in either parent population and that there is consequently a correlation between hybrid vigor and the number of dominant factors. He did not state why a greater number of dominant factors produced an increase in vigor. KEEBLE and PELLEW (1910) believed that the dominance of characters contributed by both parents might explain the increased vigor of hybrids. They crossed two varieties of peas (*Pisum sativum*) which differed in height and the F_1 hybrids were taller than either parent. The dominance of characters in the hybrids was offered as a possible explanation. I am not able to state whether the first suggestion of the dominant hypothesis should be credited to BRUCE or KEEBLE and PELLEW, but G. H. SHULL (1911) gives the credit to BRUCE.

In discussing "The Genotypes of Maize" SHULL mentions two possible explanations of hybrid or crossbred vigor. The first, and the one which SHULL favored, gave heterozygosis credit for the increased vigor and size of the F_1 generation. In many corn crosses which gave greater vigor to the F_1 hybrids he believed that a correlation existed between the degree of vigor and the number of heterozygous characters. To quote, "I do not believe that this correlation is perfect, of course, but approximate, as it is readily conceivable that even though the general principle should be correct, heterozygosis in some elements may be without effect upon vigor, or even depressing." The second hypothesis mentioned by SHULL, for which he gives A. B. BRUCE credit, is based on the assumption that the degree of vigor depends upon the number of dominant elements present rather than the number of heterozygous elements. The following quotation explains SHULL's reaction to the two hypotheses: "Mr. A. B. BRUCE proposes a slightly different hypothesis in which the degree of vigor is assumed to depend upon the number of dominant elements present, rather than the number of heterozygous elements. While all of my data thus far are in perfect accord with my own hypothesis, and I know of no instance in which self-fertilization of a corn plant of maximum vigor has not resulted in a less vigorous progeny, it is quite possible that I have still insufficient data from which to distinguish the results expected under these two hypotheses. However, for the purpose of the present discussion, it is not necessary to decide which of these two hypotheses (if either) is correct. Both of them are based upon the view that the germ-cells produced by any plant whose vigor has been increased by crossing are not uniform, some possessing positive elements or genes not possessed by others."

EAST and HAYES (1912) give support to the heterozygosis hypothesis in reporting their results secured in tobacco crosses. These investigators make a clear distinction between dominance and the effects of heterozygosis. The following quotation is of value in order to understand fully their support of the hypothesis of heterozygosis: "The term vigor has hitherto been used with the general meaning which the biologist readily understands. We will now endeavor to show in what plant characters this vigor finds expression. It is not an easy task because of the possibility of confusing the phenomena of Mendelian dominance with the physiological effects due to heterozygosis. The confusion is due to superficial resemblance only. Dominance is the expressed potency of a character in a cross and affects the character as a whole. A morphological character, like the pods of individual maize seeds, or the products of some physiological reaction like the red color of the seed pericarp in maize, may be perfect dominance, that is, it may be developed completely when obtained from only one parent. Size characters, on the other hand, usually lack dominance or at least show incomplete dominance. The vigor of the first hybrid generation theoretically has nothing to do with these facts. This is easily demonstrated if one remembers that the increased vigor manifested as height in the F_1 generation can not be obtained as a pure homozygous segregate, which would be possible if due to dominance. Furthermore, the universality with which vigor of heterozygosis is expressed as height shows the distinction between the two phenomena. If the greater height were the expression of the meeting of two factors ($T_1t_2 \times t_1T_2$) both of which were necessary to produce the character, one could not account for the frequency of the occurrence. Nevertheless, in practice the confusion exists, and while we have considerable confidence in the conclusions drawn from our experiments we have no intention of expressing them dogmatically."

A. F. SHULL (1912) and CASTLE (1916) agree that heterosis cannot be explained satisfactorily on the basis of dominant factors. SHULL, although not taking a definite stand for either hypothesis, does state that heterozygosis is more likely to account for hybrid vigor than the presence of certain dominant genes. To quote SHULL: "The former view (heterozygosis) admits of a plausible foundation in cell physiology, and the essence of it may be extended to cases of decrease of vigor in which there is no change in genotypic constitution and which are, therefore, without the pale of either theory." CASTLE takes somewhat the same view as SHULL as will be noted in the following quotation: "Crossbreeding has, then, the same advantages over close-breeding that fertilization has over

parthenogenesis. It brings together differentiated gametes, which, reacting on each other, produce greater metabolic activity. Whether or not the uniting gametes differ by Mendelian unit-characters is probably of no consequence. That they differ chemically is doubtless the essential thing in producing hybrid vigor. Heterozygosis is mentioned merely as an evidence of such chemical difference."

EMERSON and EAST (1913) offer an objection to the hypothesis of dominance because F_2 generations do not show an unsymmetrical distribution in respect to characters in which heterosis was shown in the F_1 .

This objection was based upon their study of the inheritance of quantitative characters in maize.

JONES (1917) revived the dominance hypothesis by introducing linked factors as an explanation as to why it was very difficult to get homozygous segregates possessing the vigor so often manifest in F_1 hybrids. The knowledge that genetic linkage often occurs does in a measure answer one of the chief objections to the dominance hypothesis. This principle of linkage also answers the objection raised by EMERSON and EAST as to why F_2 generations do not show an unsymmetrical distribution in respect to many known characters. A chief difficulty of the hypothesis is the necessary supposition that genes favoring growth are so specific in nature and so few in number that their being borne on homologous chromosomes and at adjacent loci in different races of the same species could regularly result in repulsion in gametogenesis in F_1 individuals, so that hybrid vigor regularly disappears in F_2 . JONES (1918) discussing "a Mendelian interpretation of heterosis" makes the following statements:

"Whether or not dominance of factors is wholly adequate to account for all of the immediate effects of exogamy remains to be seen. The former view that dominance was not concerned at all has been maintained so insistently that I have taken the extremely opposite view in order to show that dominance at least can be held responsible for a large part of the increased development shown by hybrids. The treatment of the subject in this light has been dogmatic. That cross-fertilization may produce some effect which can never be attained in self-fertilization or asexual reproduction is still possible.

"The difference between the two hypotheses is not as great as might seem at first sight. The older hypothesis is general in its application and does not commit itself to the interpretation of specific effects. The view presented here is specific in its application and may be shown to be inadequate for the interpretation of all phases of the problem of increased development following cross-fertilization.

"The greatest progress in our knowledge of inbreeding and crossbreeding was made when their effects were linked with Mendelian phenomena. This was the big step forward. The two ways of interpreting these results here differ only in minor features and it is not putting the matter fairly to hold them up as rival hypotheses, one to be chosen from the other. Placing the effects of inbreeding and crossbreeding entirely on a Mendelian basis is merely an outgrowth of the older view as knowledge of the methods of inheritance increased."

EAST and JONES (1919) agree that the dominance hypothesis explains heterosis, and state that the recent developments in our knowledge of hereditary factors, especially linkage conditions, remove the major objections to such a hypothesis. In other words, EAST has abandoned the heterozygosis hypothesis in favor of the dominance hypothesis. HAYES has also been converted to the latter hypothesis as indicated in the text on "Breeding Crop Plants" by HAYES and GARBER (1927).

WRIGHT (1922) makes the following statements in connection with results of inbreeding and crossbreeding of guinea pigs:

"Analysis of the various crosses indicates that the results are all the direct or indirect consequence of the Mendelian mechanism of heredity. The fundamental effect of inbreeding is the automatic increase in homozygosis in all respects. An average decline in vigor is the consequence of the observed fact that recessive factors, more extensively brought into expression by an increase in homozygosis, are more likely to be deleterious than are their dominant allelomorphs. The differentiation among the families is due to the chance fixation of different combinations of the factors present in the original heterozygous stock. Crossing results in improvement because each family in general supplies some dominant factors lacking in the others. Dominance or even imperfect dominance in each unit character is built up into a pronounced improvement over each parent stock in the complex characters actually observed.

"A certain portion of the increase in vigor of the first cross between inbred families is maintained on resuming random-mating. One half of this increase is maintained in stocks founded on two inbred lines, two-thirds in the case of three lines, three-fourths in the case of four lines, four-fifths in the case of five lines, and so on." The above quotations leave no doubt where WRIGHT stands on the two hypotheses.

DISCUSSION OF THE HYPOTHESES OF HYBRID VIGOR

The essential difference between the explanations which have been offered for hybrid vigor seems to be this: On the heterosis interpretation

hybrid vigor does not result either from dominant or from recessive genes as such, but from a union of unlike elements dominant with recessive, *dissimilarity* in content of genes between the uniting gametes producing, it is supposed, a more vigorously developing zygote.

The original suggestion that a greater content of *dominant* genes made for greater vigor seems to have been a logical outgrowth of the presence-absence concept, in which recessives were conceived as simple absence of the dominants. Naturally if a dominant contributed anything to the total vigor of the organism, the recessive would contribute less, so that, other things being equal, the most vigorous zygote would be the one containing the most dominants.

In the revival of the dominant theory by JONES, the presence-absence idea is dropped, and the conception brought forward of special (dominant) genes making for vigor in the organism. If a combination of all such genes could be secured in a homozygous state the maximum of vigor would be realized, according to JONES, and a heterozygous state of one or more such genes would add nothing to the vigor of the organism. In a recent paper (1926) he cites the KING inbred rats as an example of a presumably, completely homozygous organism possessing a maximum of vigor. Experimental observations to be presently discussed show, however, that still further vigor of development can be imparted to the KING inbred race by an outcross with a race no larger than itself.

In every such case JONES is forced to assume that the race with which a cross was made did after all contain one or more genes making for vigor which were not present in the inbred race. He might then simply say that he was mistaken in his previous estimate of the KING race. It left something yet to be desired in the way of vigor. The question is whether there has ever existed, or can be produced, an organism which would not derive additional vigor from an outcross. This is the gist of the heterosis interpretation, for or against which experimental evidence is desired.

EXPERIMENTAL STUDY OF HYBRID VIGOR

Location of Work and Animals Used

Experimental work was started at the Bussey Institution, HARVARD UNIVERSITY, Forest Hills, Boston Massachusetts in the fall of 1924. After one year's work at the Bussey Institution all necessary experimental animals were moved to the WEST VIRGINIA AGRICULTURAL EXPERIMENT STATION, Morgantown, West Virginia, where active breeding was continued until the late summer of 1927.

Three strains of rats (*Rattus Norvegicus*) were used and for convenience in keeping records they were designated as strains S_1 , S_2 , S_3 . Such symbols are used exclusively in charts, tables, and in all discussions referring to the different strains. These strains were stock rats maintained at the Bussey Institution laboratory previous to their use in my work.

The S_1 strain may be described as pink-eyed yellow rats of the known genetic composition $CCppRRHAA$. This stock had been rather closely bred for several generations although sib (brother and sister) matings had not been regularly followed. Ten rats of this strain, 8 females and 2 males, were selected from two litters for my work. These two litters were from sib matings, having the same sire and their dams being litter mates to each other and to the male. This strain proved to be vigorous and prolific throughout the experiment.

The rats of the S_2 strain are red-eyed hooded cream with the known genetic composition $CCPPrrhhaa$. This strain had been more closely inbred than the S_1 strain, having been closely inbred for several generations. The matings were known to have been sib matings for three generations. This strain was known not to be very prolific and it was necessary to select my initial animals, nine in number, from three litters. Two females and one male were selected from each litter. This strain was a handicap to the progress of the work due to small litters and also due to the failure of a number of females to produce more than one litter. I am unable to explain the fact that many females of this strain produce one litter and fail to conceive thereafter.

The rats of the S_3 strain are known as KING inbred albinos. This strain was secured from the WISTAR INSTITUTE of Philadelphia through the kindness of Doctor HELEN DEAN KING, a short time before this experiment was started. The known genetic composition of this strain is $ccPPRRhhaa$. The foundation rats of this strain consisted of six females and one male from a litter of twelve. This litter represented the fifty-first generation of brother by sister matings according to the records of Doctor KING and the Bussey Institution. This strain proved prolific and vigorous throughout the experiment.

Basis of Study

This study of hybrid vigor is based solely upon growth as measured by weight. A careful growth record was made of all stock strains (S_1 , S_2 , and S_3) and of the F_1 and F_2 progeny of crosses, $S_1 \times S_2$ and $S_1 \times S_3$. The F_2 progeny of the $S_1 \times S_2$ cross were all tested for their genetic consti-

tution by back-crossing to the stock strains, thus giving a known genetic basis for studying animals of the F_2 generation.

Methods of Handling Experimental Animals

1. Rats were kept in the standard 14"×17" wire cages which were used for all rat work at the Bussey Institution.

2. All rats were fed and cared for in as like a manner as possible.

3. With few exceptions, five rats were retained in a cage from the time they were weaned at 30 days of age until they were 90 days of age.

4. Cages and other equipment (water bottles and feed dishes) were thoroughly cleaned each week to guard against disease and digestive disturbances.

5. All rats were ear marked and careful growth weights to the nearest 5 grams were recorded for 30, 50, 70, 90 and 150 days of age.

6. A Hanson Brothers' spring scale, graduated to one gram and weighing a maximum of 500 grams, was used for weighing the rats.

7. All stock rats (S_1 , S_2 , and S_3) were produced by brother-sister matings during the experiment.

8. In all cases total litters were recorded and the only rats born in litters contained in this work, not accounted for, died before their growth records were complete, or failed to breed. Apparent sterility in a few F_2 ($S_1 \times S_2$) rats eliminated them from studies involving their genetic constitution.

9. All statistical studies are based on the weights of rats at 90 days of age. Many females were mated following the 90 day weight period and the subsequent occurrence of gestation and lactation periods, change of cages, and the varying number of animals kept in one cage make it undesirable to use the weights of the 150 day period.

DATA AND STATISTICAL STUDIES

Average Weights

Males and females of each parent strain and of their F_1 and F_2 progenies were treated separately to get the average weight of each sex at 30, 50, 70, 90, and 150 days of age. Table 1 shows these average weights and the number of rats each group contains. This table does not contain all rats used in this study, as many stock rats were used for breeding purposes in connection with tests to determine the genetic constitution of F_2 ($S_1 \times S_2$) rats. For such test animals growth weights were not recorded. The S_3 strain of rats gave so much trouble, due to small litters and their failure

to breed, that the progeny of the cross between the S_1 and S_2 strains were not studied extensively. However, the data derived from this cross are of interest in connection with the data of the other cross ($S_1 \times S_3$). The data contained in table I are given graphically in figures 1-4.

TABLE 1

Average weight in grams of rats of various stocks and of their F_1 and F_2 progeny at ages 30-150 days.

STOCK	SEX	NO	30 DAYS	50 DAYS	70 DAYS	90 DAYS	150 DAYS
S_1	Males	100	43	86	132	168	234
S_1	Females	98	44	81	111	134	175
S_2	Males	52	30	58	100	130	189
S_2	Females	59	28	54	79	100	137
S_3	Males	62	46	99	158	199	268
S_3	Females	80	45	87	125	153	199
$F_1(S_1 \times S_2)$	Males	48	47	101	159	206	272
$F_1(S_1 \times S_2)$	Females	37	44	92	129	156	191
$F_2(S_1 \times S_2)$	Males	51	46	97	150	183	247
$F_2(S_1 \times S_2)$	Females	64	42	83	116	141	179
$F_1(S_1 \times S_3)$	Males	53	44	117	179	213	278
$F_1(S_1 \times S_3)$	Females	69	43	97	141	161	205
$F_2(S_1 \times S_3)$	Males	137	42	87	133	177	244
$F_2(S_1 \times S_3)$	Females	132	41	81	114	142	179

Growth Curves

By a glance at figures 1 and 3, one is convinced that heterosis is exhibited in F_1 animals of each cross made. Figures 2 and 4 show that this F_1 vigor is materially reduced in the F_2 generation of each cross. In the case of the S_1 by S_2 cross (figure 1) there is a large difference in size between the parent strains. The average weight of S_1 males is 234 grams and the average weight of S_2 males is 189 grams at 150 days of age, yet the F_1 males have an average weight greater than that of males of the heavier parent race (S_1), namely 272 grams at 150 days of age. The same relation exists in the case of the F_1 females compared with the parent stocks. Figure 2 shows that both the F_2 males and the F_2 females fail to develop as rapidly and are smaller at 150 days of age than the same sex of the F_1 generation. Even though a noticeable decrease in size is shown in the F_2 generation, both males and females are still heavier at 150 days of age than the males and females of the larger or S_1 parent strain. In

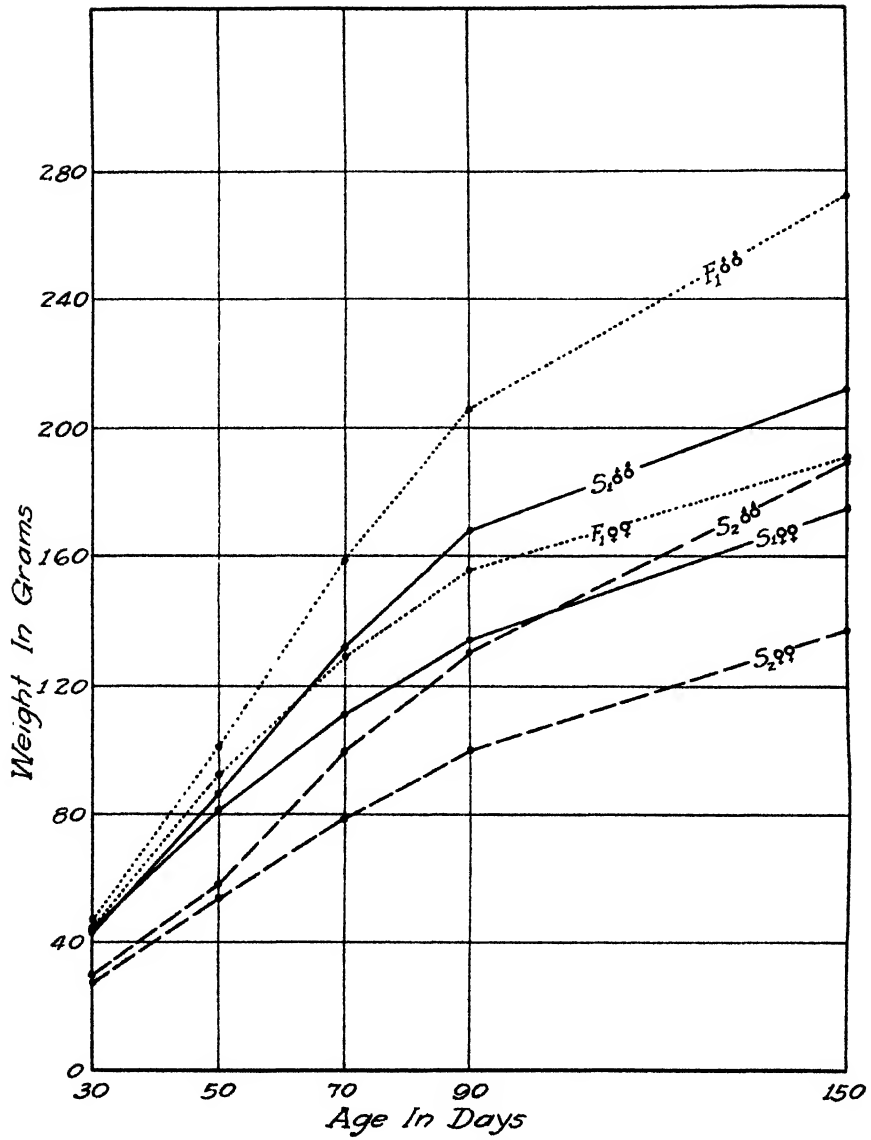


FIGURE 1.—Growth curves for S_1 and S_2 stock and their F_1 progeny.

this cross S_2 males were, in all cases, mated to S_1 females due to the low productiveness of the S_2 females.

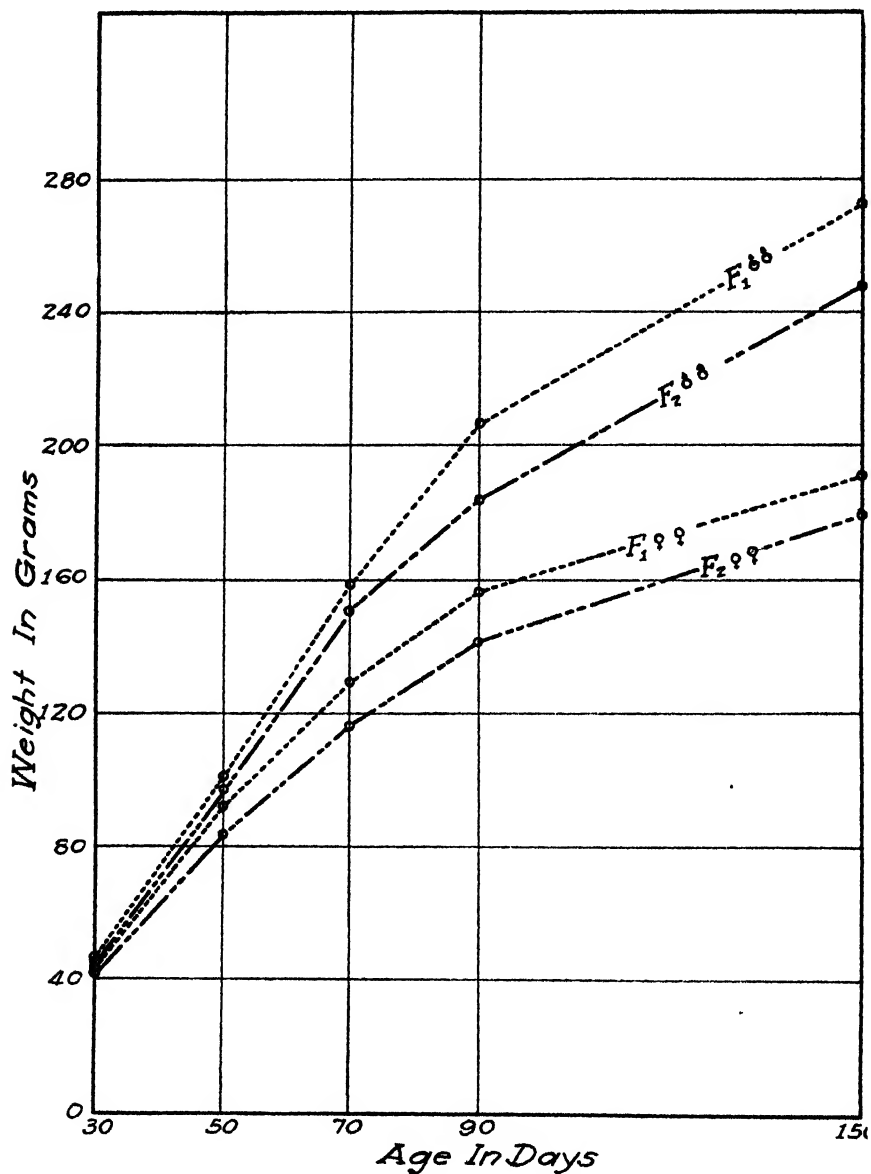


FIGURE 2.—Growth curves for F_1 and F_2 progeny of $S_1 \times S_2$ stocks.

The stock strains of the S_1 by S_3 cross, as shown in figure 3, are nearer the same size at 150 days of age than were the stock strains (S_1 and S_2)

of the previous cross. The results of the S_1 by S_3 cross are comparable to the results of the S_1 by S_2 cross, with one exception; the F_2 generation

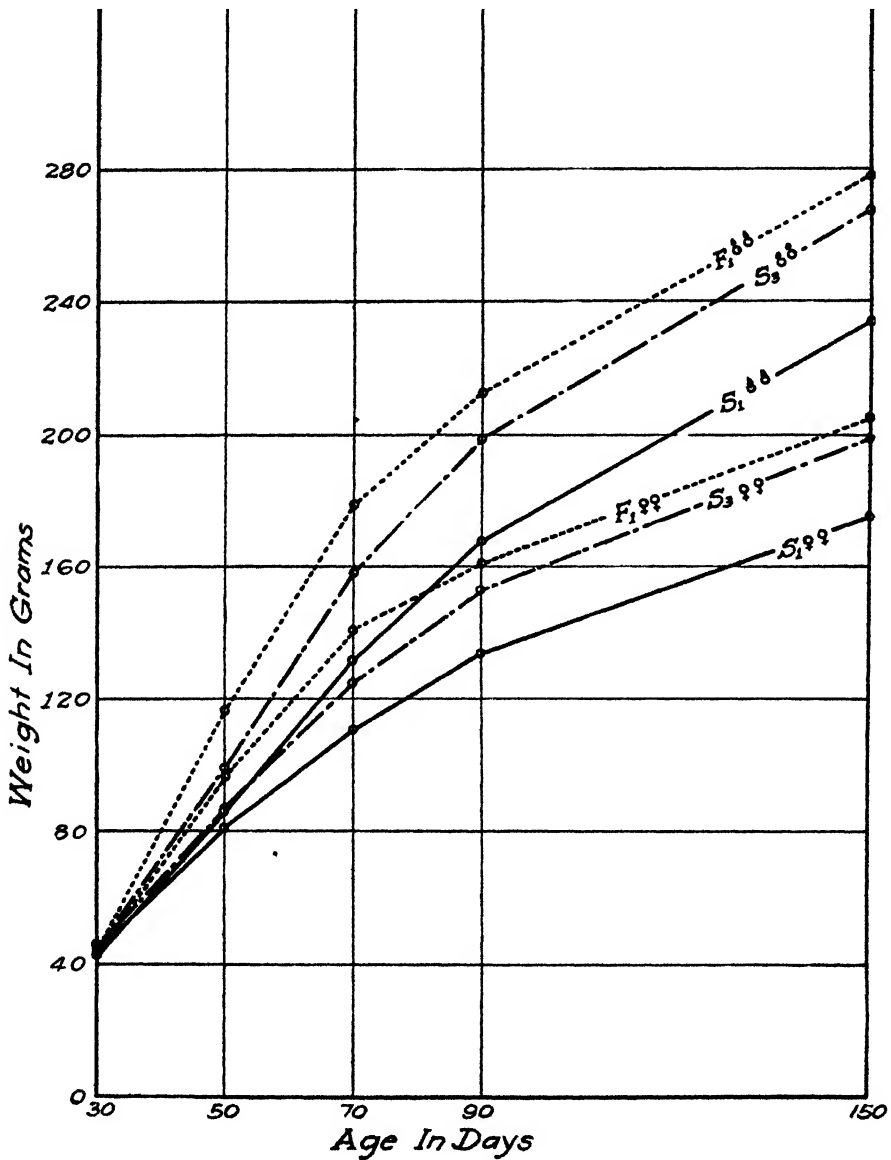


FIGURE 3.—Growth curves for S_1 and S_3 stock and their F_1 progeny.

(figure 4) drops in average weight to near the average weight of the smaller or S_1 parent strain at the same age. In this case the F_1 vigor apparently

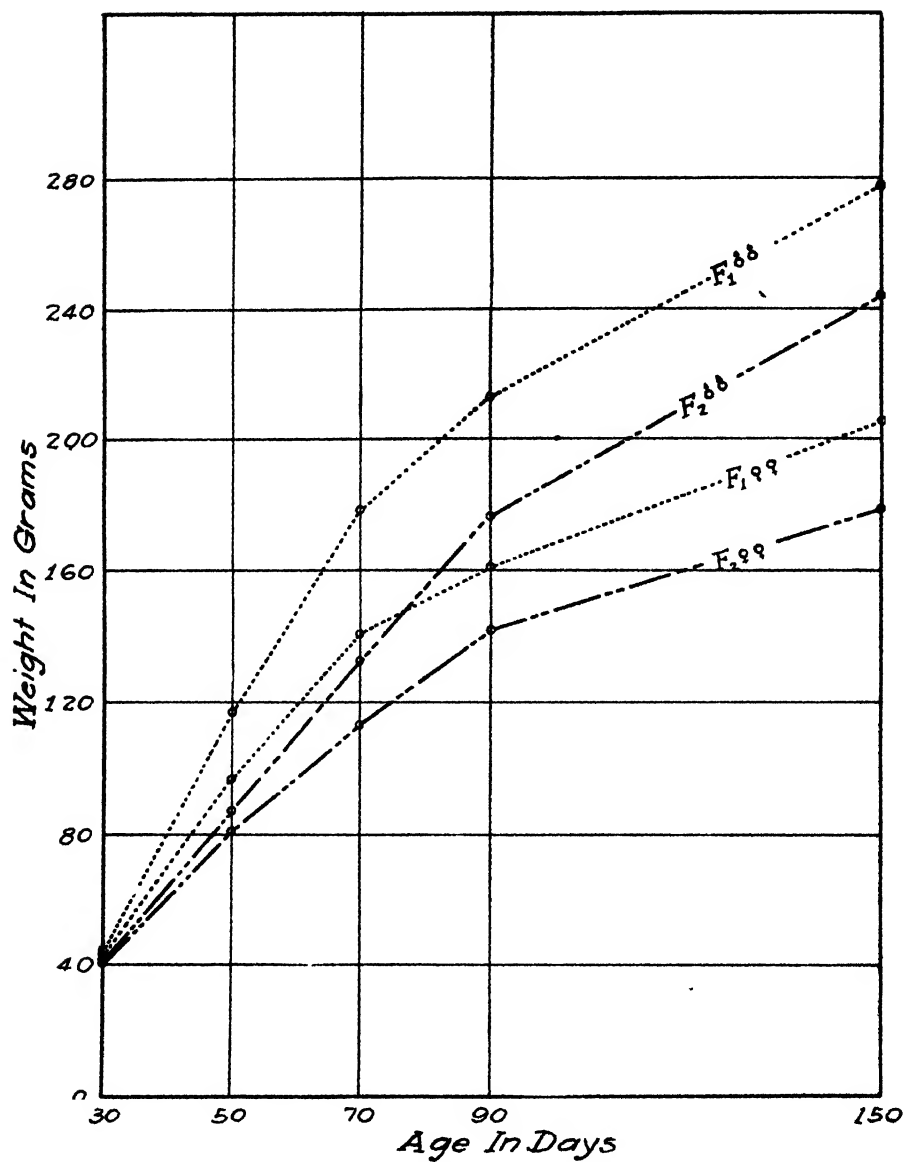


FIGURE 4.—Growth curves for F_1 and F_2 progeny of $S_1 \times S_2$ stock.

was being lost at a more rapid rate than in the former cross. The average loss in weight between the F_1 and F_2 generations, at 150 days of age, in the two crosses is as follows:

	$S_1 \times S_2$	$S_1 \times S_3$
Loss in weight of males	25 grams	34 grams
Loss in weight of females	12 grams	26 grams

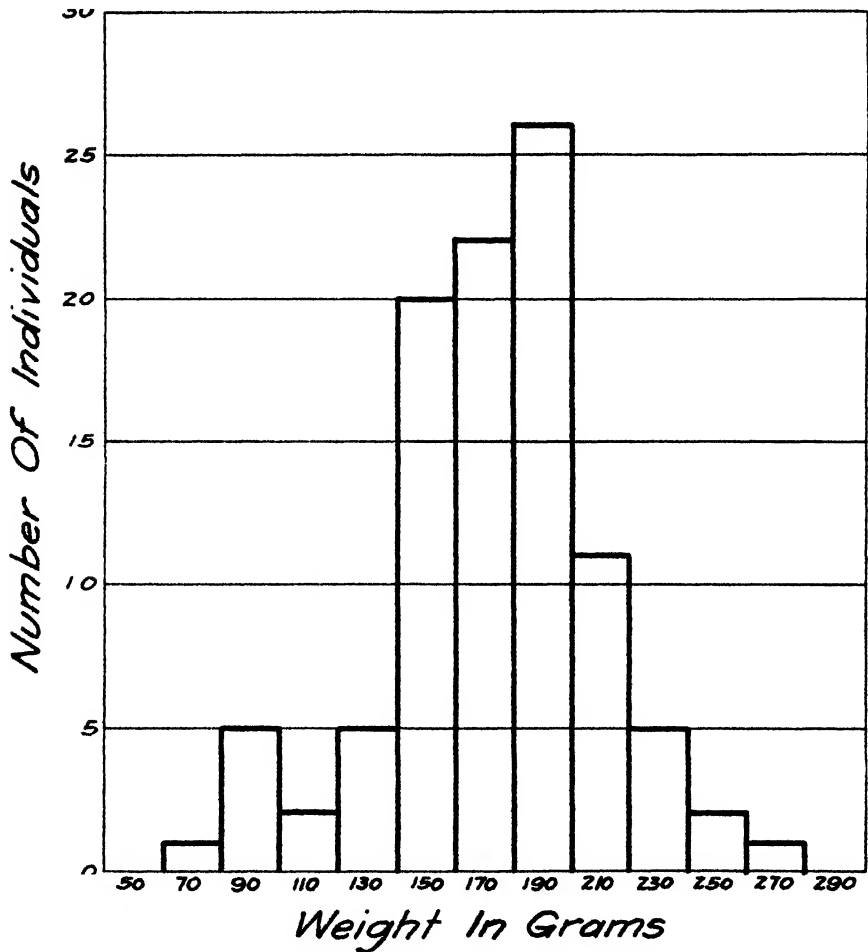


FIGURE 5.—Frequency polygon showing variation in weight of 100 S_1 males at 90 days of age.

Frequency Polygons

Frequency polygons (figures 5–12) show the variations in weight at ninety days of age of all male and all female rats in connection with the

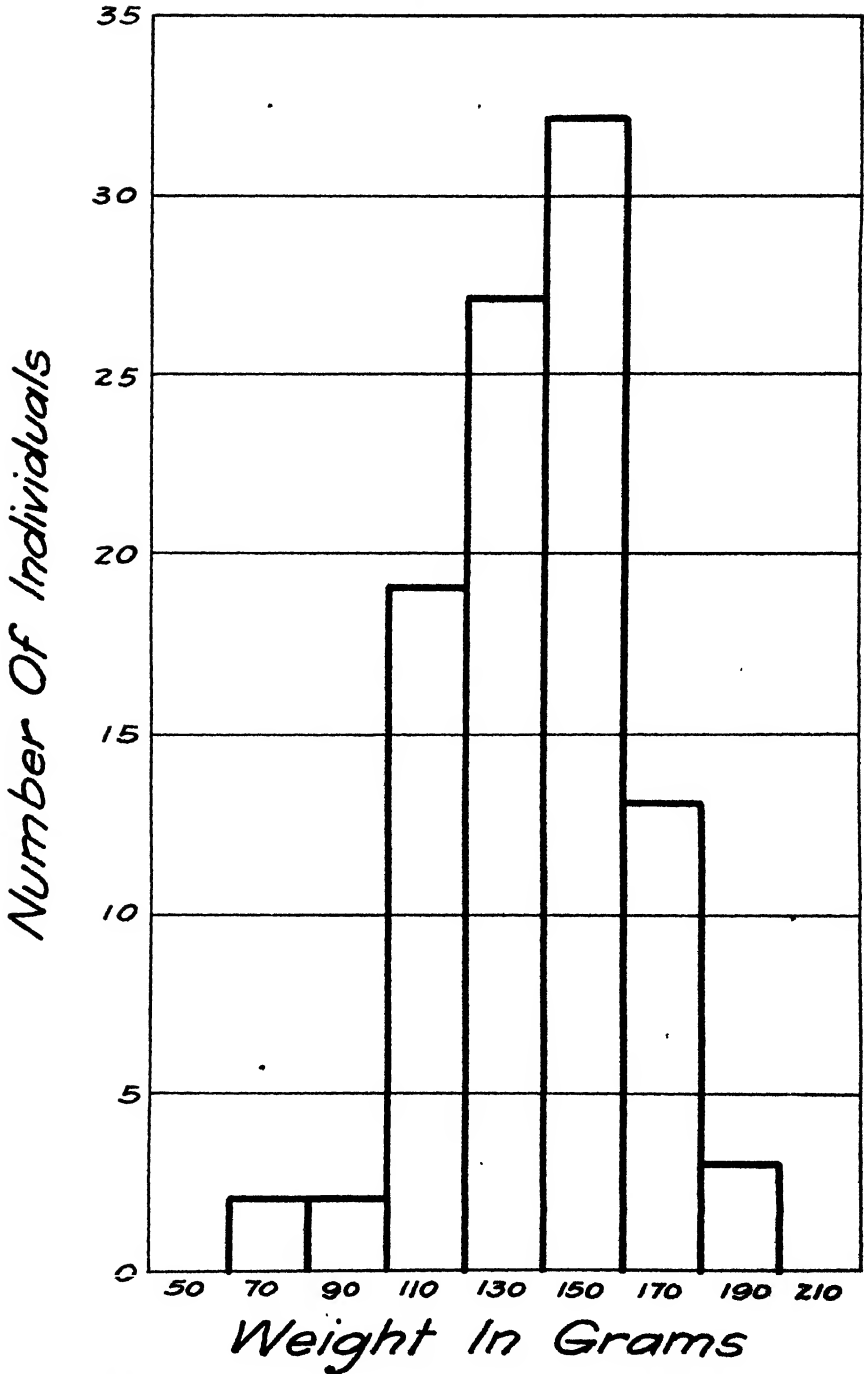


FIGURE 6.—Frequency polygon showing variation in weight of 86 S_1 females at 90 days of age.

$S_1 \times S_2$ cross. These graphs show that male rats are more variable than females in the S_1 and S_2 stock strains. The F_1 and the F_2 rats do not show any marked difference in the variability of males and females. However, table 2 shows that the coefficient of variability for the F_1 females is slightly higher than for the F_1 males, and that the coefficients of variability of the F_2 males and females are almost identical. Figures 9 and 11 show slightly more variability among the F_2 males than among the F_1 males,

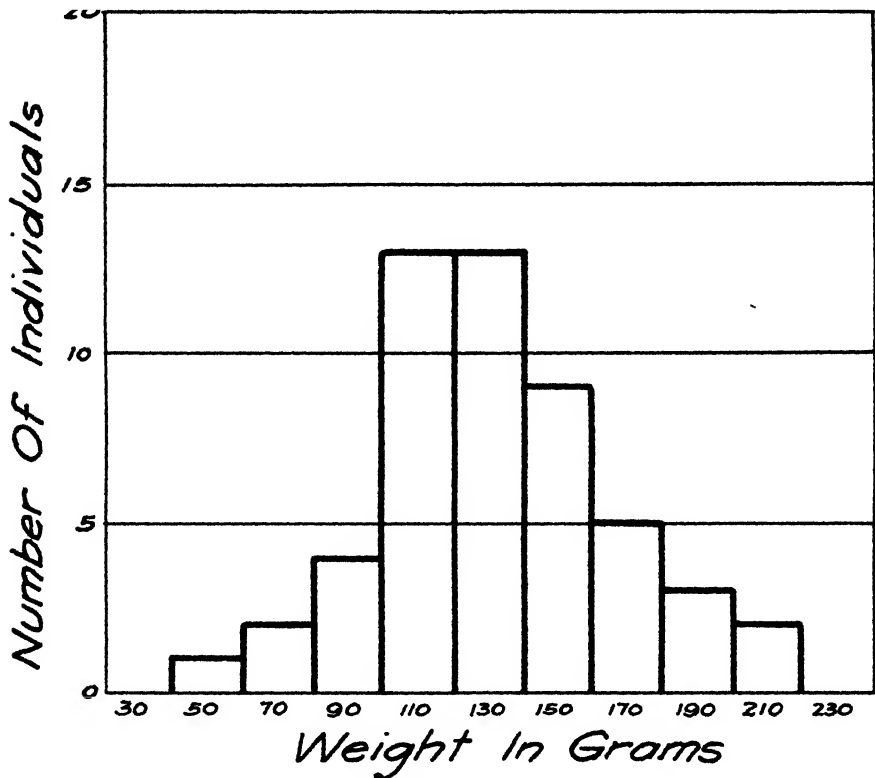


FIGURE 7.—Frequency polygon showing variation in weight of 52 S_2 males at 90 days of age.

but the F_2 males are less variable than the males of either parent strain (figures 5 and 7). Figures 10 and 12 do not indicate a greater variability among the F_2 females than among the F_1 females. In fact, these graphs indicate less variability among the F_2 females. Table 2 shows the coefficients of variability for the F_1 and F_2 females to be almost identical.

Frequency polygons (figures 5, 6, and 13–18) show the variations in weight of all male and all female rats in connection with the S_1 by S_2

cross, at 90 days of age. These graphs indicate that male rats are uniformly more variable than females of the same stock strain or the same progeny generation. This greater variability of the body weight of male rats was observed also by KING (1923). These graphs also indicate that

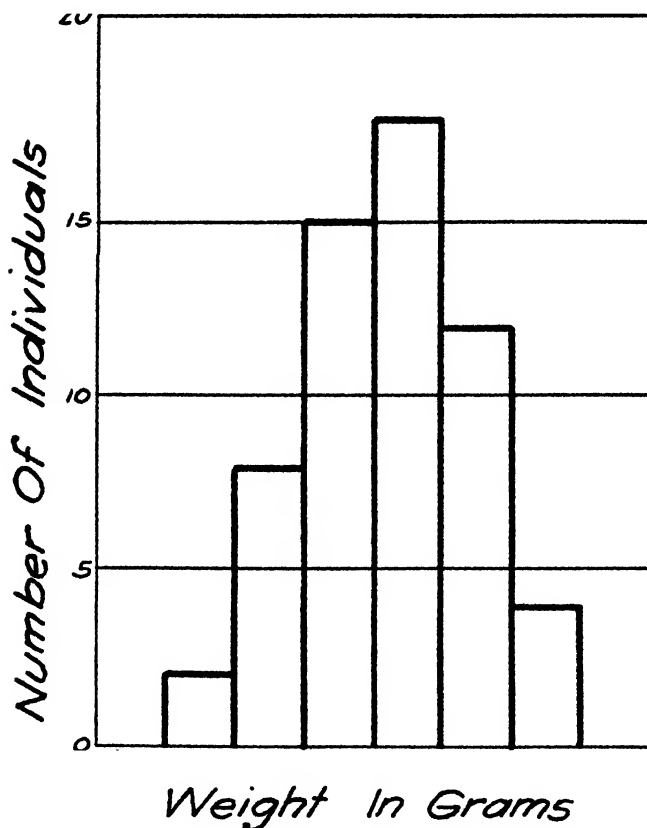


FIGURE 8.—Frequency polygon showing variation in weight of 59 S_2 females at 90 days of age.

both F_1 males and F_1 females are less variable than the corresponding sex of either parent strain. Figures 15 and 17 indicate a greater variability among F_2 males than among F_1 males, but this relation does not exist in the case of F_1 and F_2 females as shown in figures 16 and 18.

Statistical Study at Ninety Days of Age

Growth curves and frequency polygons are valuable graphical helps to visualize the comparative rate of growth and the comparative variations of the groups of rats studied, but they do not give a positive answer as to whether the difference in growth or the difference in variation is large

enough to be significant. Therefore a more reliable statistical study has been attempted as shown in tables 2, 3, and 4. The data contained in table 2 are based upon the weights of rats at ninety days of age. I have previously mentioned why I selected weights at this age for this study.

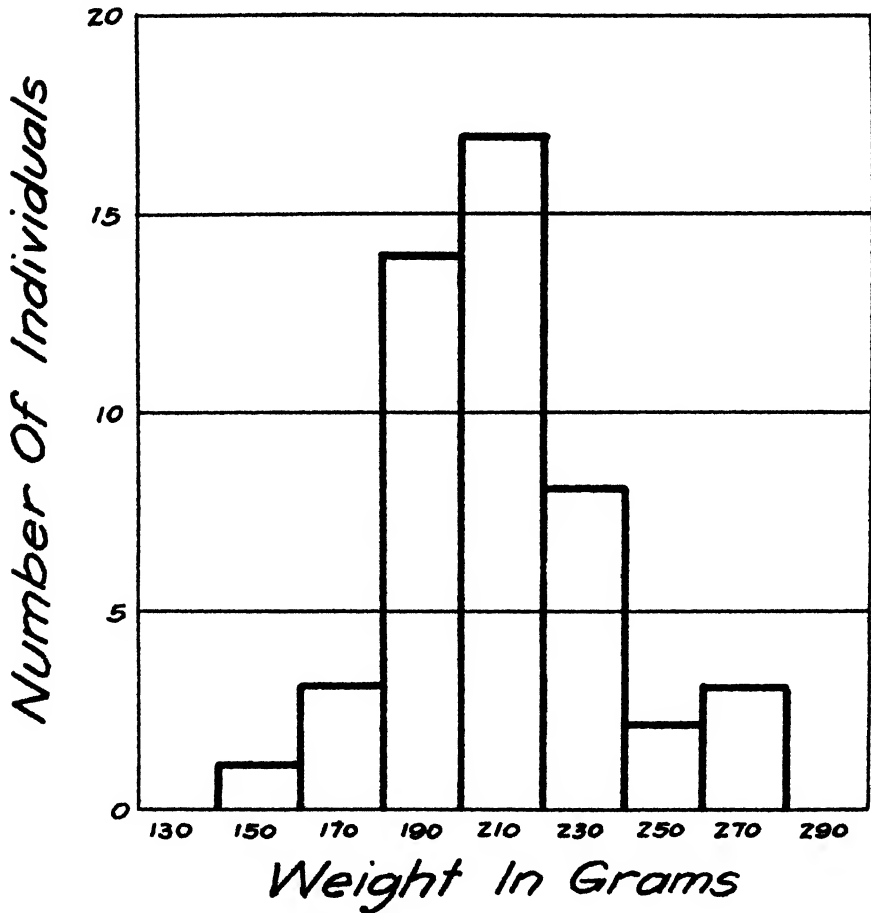


FIGURE 9.—Frequency polygon showing variation in weight of 48 F_1 ($S_1 \times S_2$) males at 90 days of age.

The data of table 2 were calculated on the same frequency as previously shown in frequency polygons.

Tables 3 and 4 are based on the data contained in table 2 and show in a comparative way whether the differences in mean weights and the differences in coefficients of variability are significant or not. In making these

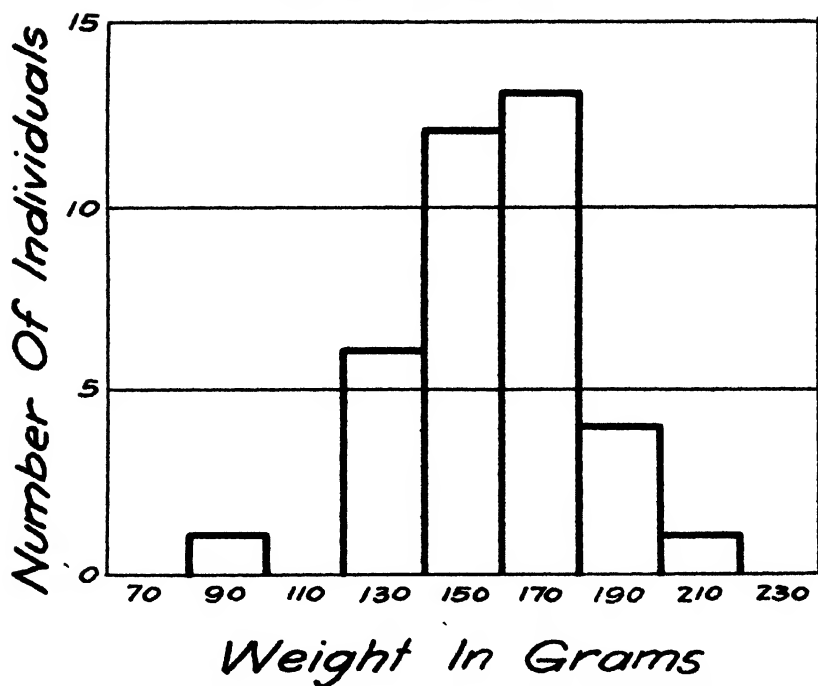


FIGURE 10.—Frequency polygon showing variation in weight of 37 F₁ (S₁ × S₂) females at 90 days of age.

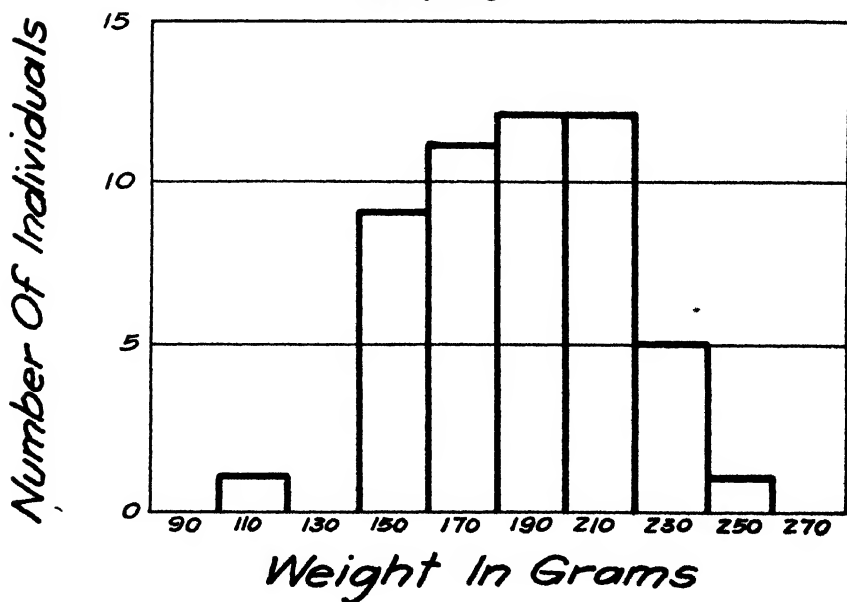


FIGURE 11.—Frequency polygon showing variation in weight of 51 F₂ (S₁ × S₂) males at 90 days of age.

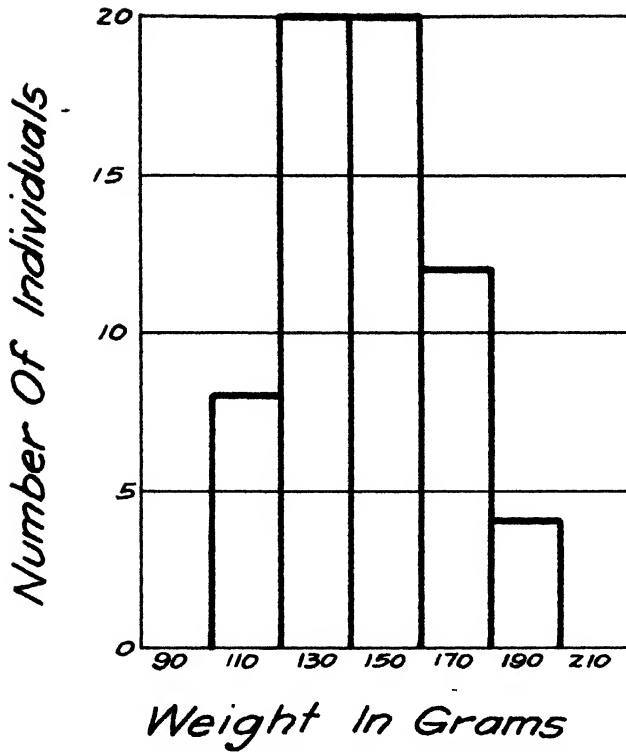


FIGURE 12.—Frequency polygon showing variation in weight of 64 F₂ (S₁ × S₂) females at 90 days of age.

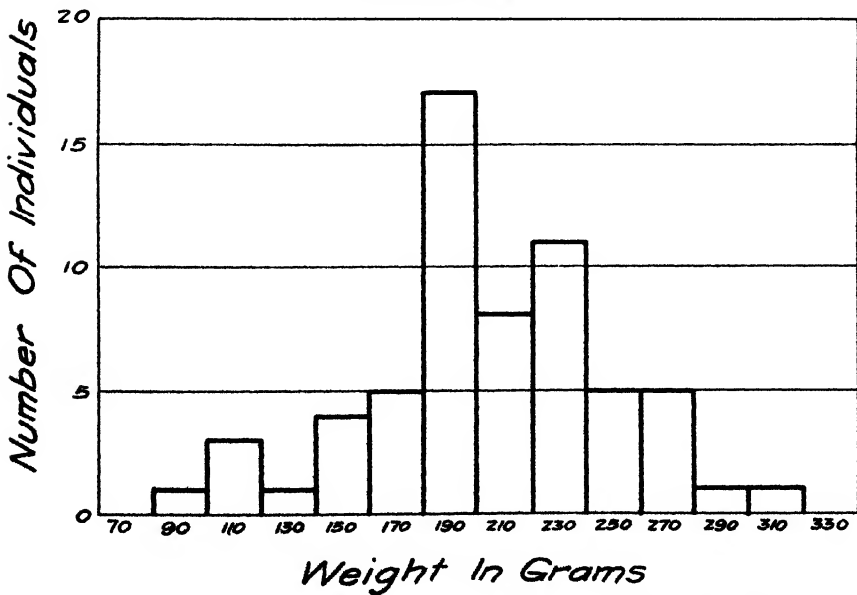


FIGURE 13.—Frequency polygon showing variation in Weight of 63 S₃ males at 90 days of age.

comparisons I used the following formula in calculating the probable error of the differences:

$$\text{Probable error of difference} = \sqrt{\sum (\text{p.e.})^2}$$

Table 3 contains the comparative data on the difference in mean weights and the difference in the coefficient of variability of groups of rats connected with the $S_1 \times S_2$ cross. These data show that both the S_1 males and the S_1 females are significantly larger than the same sex of the S_2 strain;

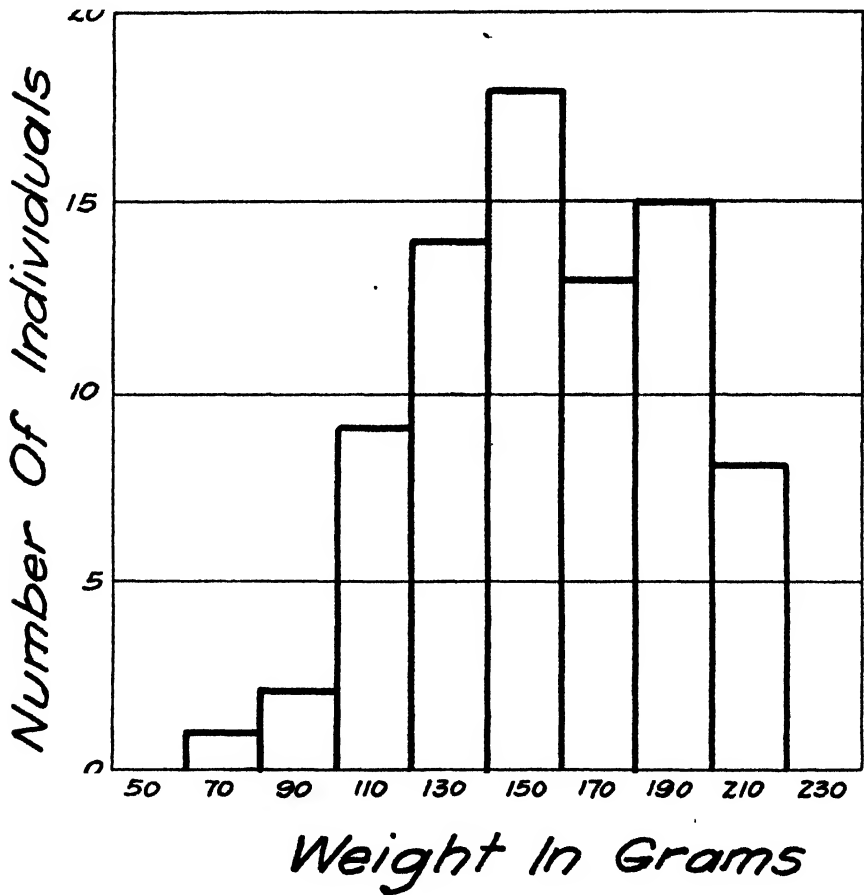


FIGURE 14.—Frequency polygon showing variation in weight of 80 S_2 females at 90 days of age.

that the S_2 females are significantly more variable than the S_1 females; and that while the S_1 males are more variable than the S_2 males, it is not a significant difference. In comparing the S_1 and S_2 strains with the F_1 generation we find both males and females of the F_1 generation signifi-

cantly larger and less variable than the corresponding sexes of the S_1 and S_2 strains. The F_1 rats of either sex are larger than the F_2 rats of the same sex, and while the F_2 rats are slightly more variable than the F_1 's the difference is so slight that it is not significant. In comparing the size and variability of the F_2 generation with the parent strains, we find the F_2 rats to be significantly larger than the larger parent strain and less variable

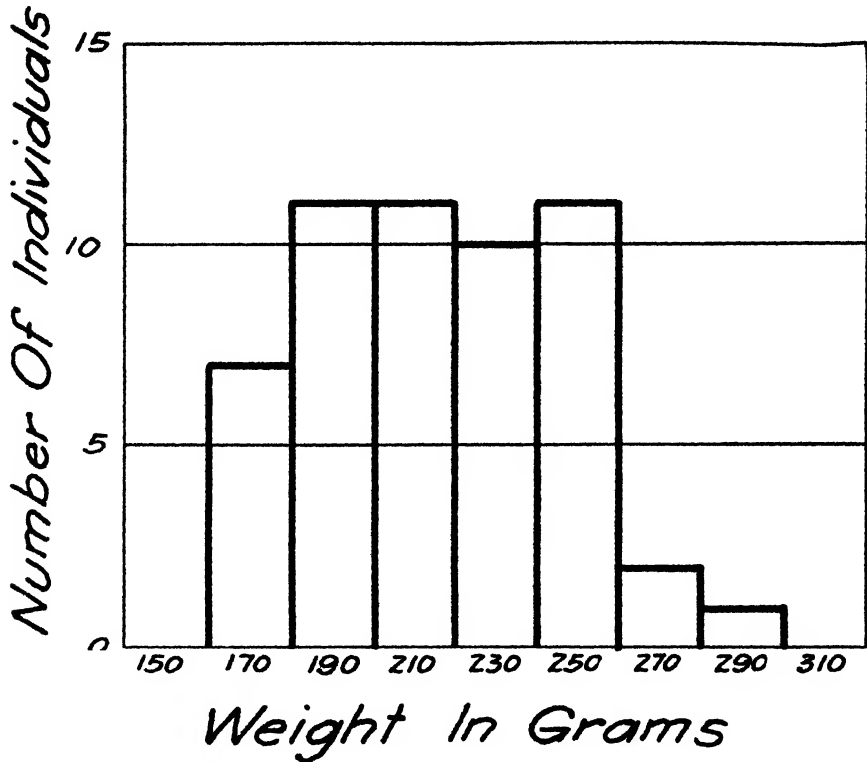


FIGURE 15.—Frequency polygon showing variation in weight of 53 F_1 ($S_1 \times S_2$) males at 90 days of age.

than either parent strain. This difference in variability, however, is not significant in the case of the S_1 and F_2 females.

Table 4 contains the comparative data on the difference in the mean weights and coefficients of variability of groups of rats connected with the S_1 by S_2 cross. These data show that both males and females of the S_2 strain are significantly larger than the males and females of the S_1 strain, and that there is not a large difference in the variability of the two

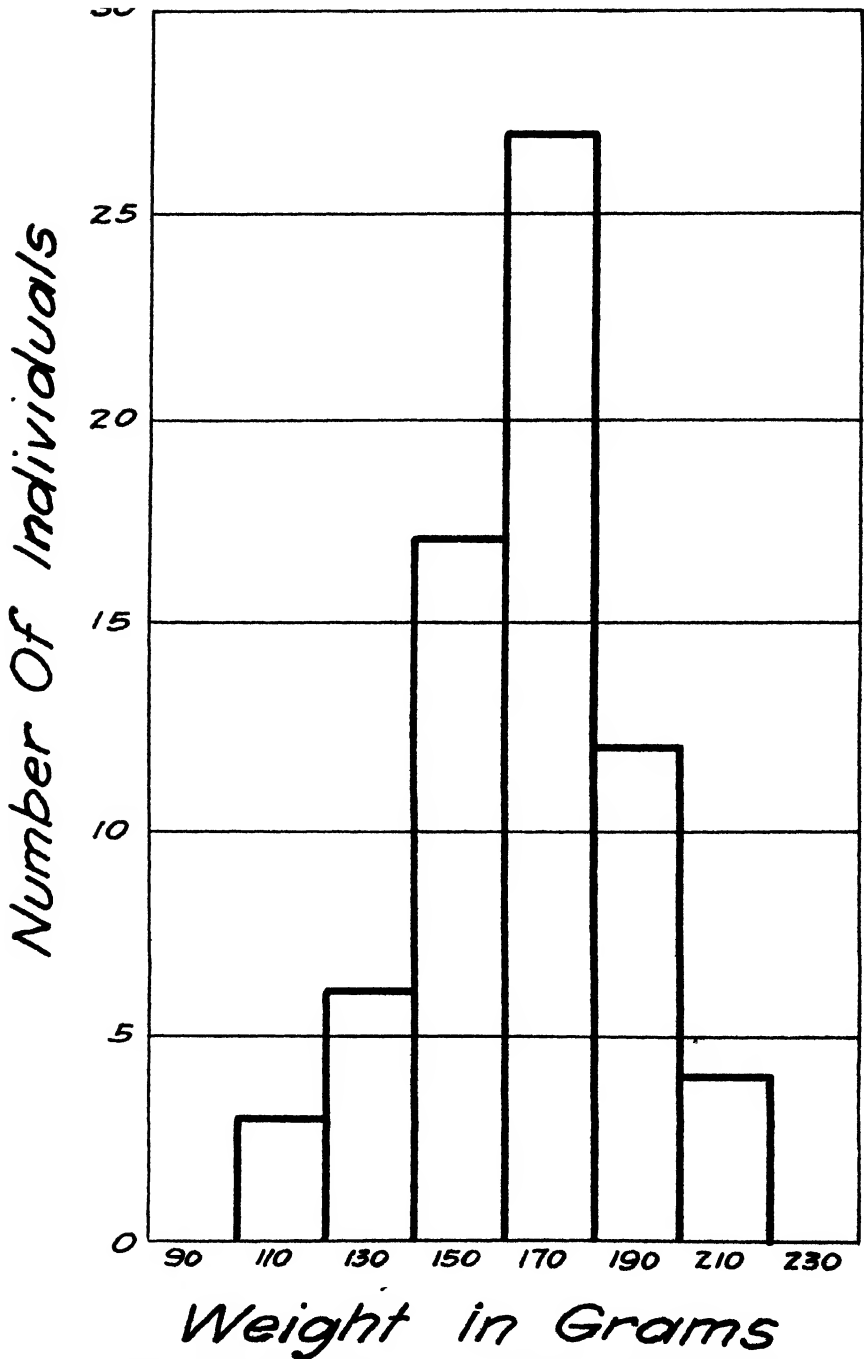


FIGURE 16.—Frequency polygon showing variation in weight of 69 $F_1(S_1 \times S_2)$ females at 90 days of age.

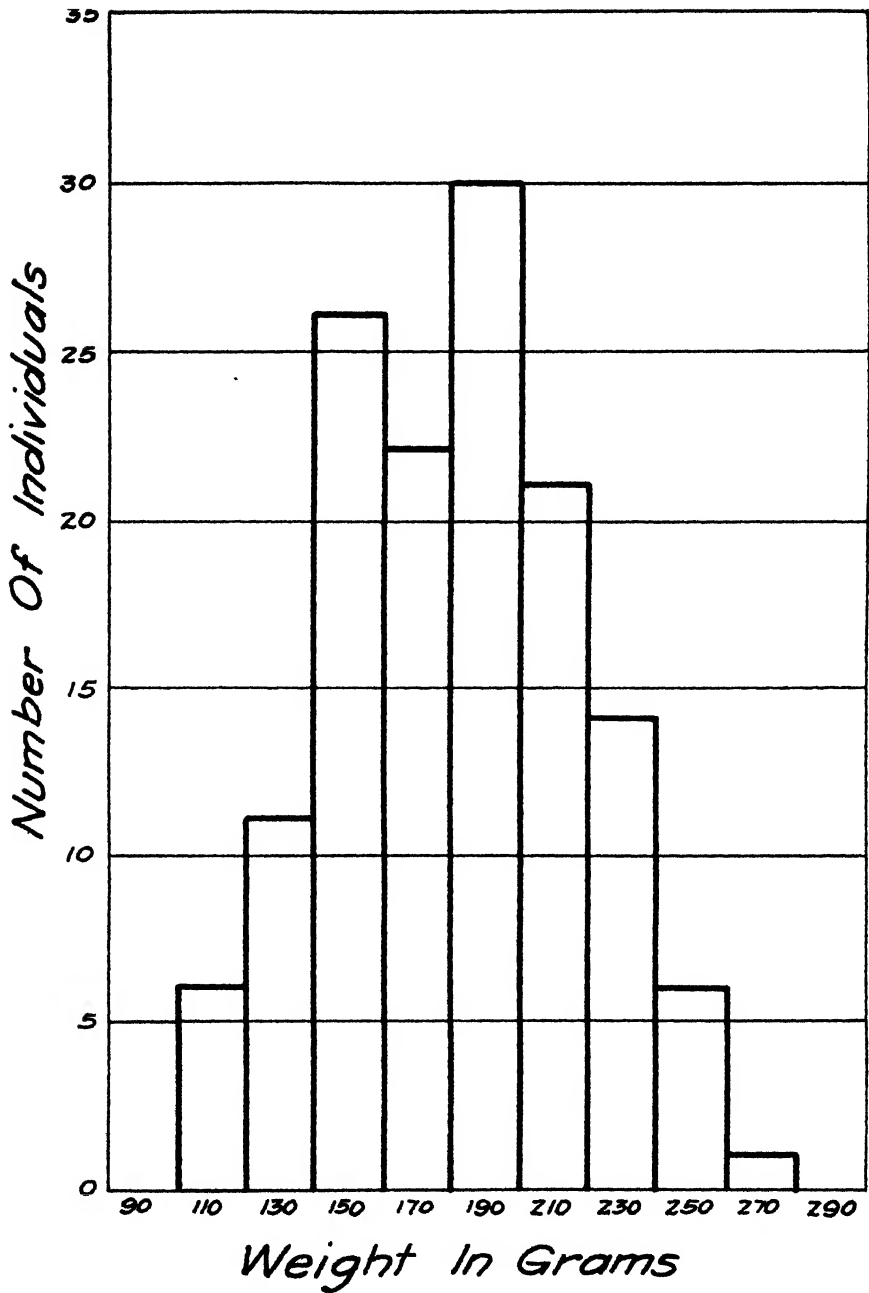


FIGURE 17.—Frequency polygon showing variation in weight of 137 F₂ (S₁ × S₄) males at 90 days of age.

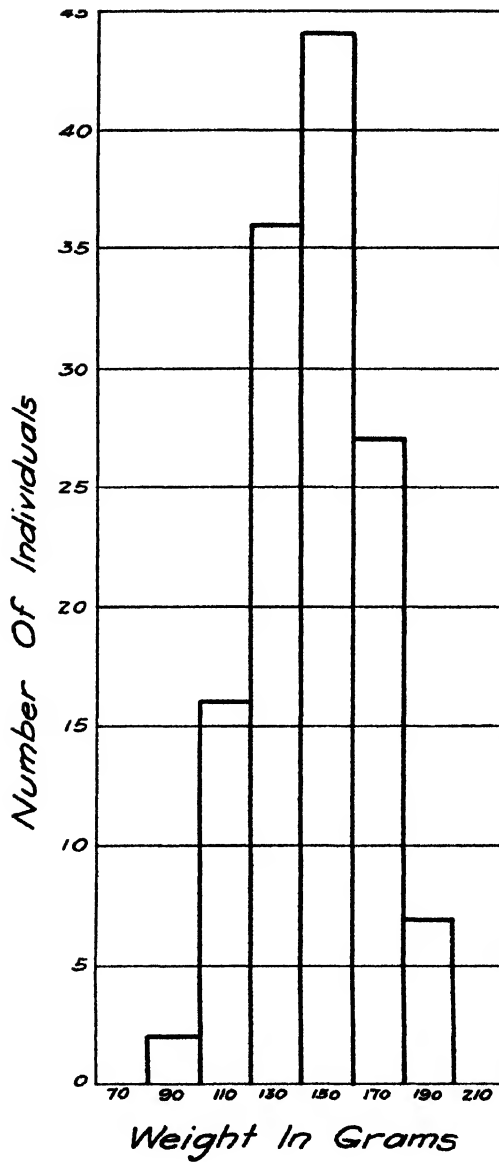


FIGURE 18.—Frequency polygon showing variation in weight of 132 $F_2(S_1 \times S_6)$ females at 90 days of age.

TABLE 2

Statistical study of weights of rats of strains S_1 , S_2 , and S_3 , and of their F_1 and F_2 progeny at age 90 days.

STOCK	SEX	NO	M	S	C. OF V.
S_1	Males	100	173.0 ± 3.17	47.02 ± 2.24	27.17 ± 1.29
S_1	Females	98	137.8 ± 1.64	24.03 ± 1.16	17.43 ± 0.84
S_2	Males	52	131.9 ± 3.18	34.01 ± 2.24	25.78 ± 1.70
S_2	Females	59	104.3 ± 2.14	24.43 ± 1.51	23.43 ± 1.45
S_3	Males	62	203.9 ± 3.98	44.80 ± 2.70	21.95 ± 1.33
S_3	Females	80	156.3 ± 2.52	34.44 ± 1.77	21.30 ± 1.13
$F_1(S_1 \times S_2)$	Males	48	209.2 ± 2.48	25.47 ± 1.75	12.12 ± 0.83
$F_1(S_1 \times S_2)$	Females	37	159.2 ± 2.55	23.06 ± 1.81	14.48 ± 1.13
$F_1(S_1 \times S_3)$	Males	53	218.4 ± 2.85	29.95 ± 2.02	13.71 ± 0.95
$F_1(S_1 \times S_3)$	Females	69	164.3 ± 1.65	19.49 ± 1.16	11.86 ± 0.71
$F_2(S_1 \times S_2)$	Males	51	186.9 ± 2.40	28.39 ± 1.69	15.18 ± 1.01
$F_2(S_1 \times S_2)$	Females	64	145.0 ± 1.83	21.79 ± 1.30	15.02 ± 0.89
$F_2(S_1 \times S_3)$	Males	137	181.3 ± 2.02	35.01 ± 1.42	19.31 ± 0.79
$F_2(S_1 \times S_3)$	Females	132	145.0 ± 1.31	22.34 ± 0.93	15.40 ± 0.64

The mean weights are slightly larger in this table than the average weights at 90 days of age as shown in table 1. The value of frequencies was made slightly higher by uniformly throwing all weights which fell on group weight divisions into the higher groups.

strains of rats. In comparing the stock strains, S_1 and S_3 , with their F_1 progeny we find that the F_1 rats are larger than either parent strain. However, the difference in size is not large enough in the case of the S_3 and F_1 females to indicate a positive difference in size. In comparing the F_1 and F_2 rats we find that the rats of the F_1 generation are larger and less variable than the F_2 's. In comparing stock rats with the F_2 generation, the data show that the F_2 rats are intermediate in size between the two stock strains and less variable than either of the stock strains.

Known Genes not Linked with Growth Genes

Since there is a higher coefficient of variability with respect to weight among the F_2 rats than among the F_1 rats of the $S_1 \times S_3$ cross, it should be of interest to group the F_2 rats in a manner which might show whether there is any major growth gene linked with the known genes studied. It should be kept in mind that only three pairs of chromosomes are involved in this study.

TABLE 3

Comparison of means and coefficients of variability in S₁ and S₂ stocks and in their F₁ and F₂ progeny.

	DIFFERENCE IN MEAN WEIGHT	LARGER	DIFFERENCE IN C. OF V	MORE VARIABLE
S ₁ and S ₂ Males	41.1 ± 4.48	S ₁	1.38 ± 2.13	S ₁ (N.S.)
S ₁ and S ₂ Females	33.5 ± 2.69	S ₁	6.00 ± 1.66	S ₂
S ₁ and F ₁ Males	36.2 ± 4.02	F ₁	15.05 ± 1.53	S ₁
S ₁ and F ₁ Females	21.4 ± 3.02	F ₁	15.27 ± 1.81	S ₁
S ₂ and F ₁ Males	77.3 ± 4.03	F ₁	13.67 ± 1.89	S ₂
S ₂ and F ₁ Females	54.9 ± 3.32	F ₁	8.95 ± 1.83	S ₂
F ₁ and F ₂ Males	22.3 ± 3.45	F ₁	3.06 ± 1.30	F ₂ (N.S.)
F ₁ and F ₂ Females	14.2 ± 3.13	F ₁	0.54 ± 1.43	F ₂ (N.S.)
S ₁ and F ₂ Males	13.9 ± 3.97	F ₂	11.99 ± 1.63	S ₁
S ₁ and F ₂ Females	7.2 ± 2.45	F ₂	2.41 ± 1.22	S ₁ (N.S.)
S ₂ and F ₂ Males	55.0 ± 3.98	F ₂	10.61 ± 1.97	S ₂
S ₂ and F ₂ Females	40.7 ± 2.81	F ₂	8.41 ± 1.70	S ₂

N. S. as used in tables 3 and 4 indicates "not significant."

TABLE 4

Comparison of means and coefficients of variability in S₁ and S₂ stocks and in their F₁ and F₂ progeny.

	DIFFERENCE IN MEAN WEIGHT	LARGER	DIFFERENCE IN C. OF V.	MORE VARIABLE
S ₁ and S ₂ Males	30.9 ± 5.09	S ₂	5.22 ± 1.79	S ₁
S ₁ and S ₂ Females	18.5 ± 3.00	S ₂	3.96 ± 1.40	S ₂ (N.S.)
S ₁ and F ₁ Males	45.4 ± 4.25	F ₁	13.46 ± 1.60	S ₁
S ₁ and F ₁ Females	26.5 ± 2.32	F ₁	5.57 ± 1.09	S ₁
S ₂ and F ₁ Males	14.5 ± 4.98	F ₁	8.24 ± 1.62	S ₂
S ₂ and F ₁ Females	8.0 ± 3.00	F ₁ (N.S.)	9.53 ± 1.33	S ₂
F ₁ and F ₂ Males	37.1 ± 3.48	F ₁	5.60 ± 1.23	F ₂
F ₁ and F ₂ Females	19.3 ± 2.10	F ₁	3.54 ± 0.99	F ₂
S ₁ and F ₂ Males	8.3 ± 3.75	F ₂ (N.S.)	7.86 ± 1.50	S ₁
S ₁ and F ₂ Females	7.2 ± 2.09	F ₂	2.03 ± 1.04	S ₁ (N.S.)
S ₂ and F ₂ Males	22.6 ± 4.45	S ₂	2.64 ± 1.54	S ₂ (N.S.)
S ₂ and F ₂ Females	11.3 ± 2.83	S ₂	5.99 ± 1.32	S ₂

In crossing the S_1 ($CCppRRHHAA$) and S_2 ($ccPPRRhhaa$) stock strains we obtain F_1 rats which are agouti in color and of the genetic constitution $CcHhAa$. In the F_2 generation we get all the possible combinations of these three allelomorphic pairs of genes; therefore, if there are major growth genes carried on the same chromosomes, they should be brought to light by the F_2 groupings made in table 5. The data of table 5 do not give any definite evidence that growth genes are linked with the known

TABLE 5
Statistical study of weights of F_2 animals, $S_1 \times S_2$ cross, at 90 days of age.

SEX	GENES	NUMBER OF RATS	<i>M</i>	<i>S. D.</i>	<i>C. OF V.</i>
Males	<i>CC</i>	45	185.1 \pm 3.11	31.02 \pm 2.20	16.97 \pm 1.20
"	<i>Cc</i>	60	181.3 \pm 3.24	37.29 \pm 2.29	15.05 \pm 0.92
"	<i>cc</i>	36	169.5 \pm 3.87	34.47 \pm 2.74	20.33 \pm 1.61
"	<i>HH</i>	51	168.5 \pm 3.05	32.38 \pm 2.16	19.21 \pm 1.28
"	<i>Hh</i>	62	190.7 \pm 3.27	38.24 \pm 2.32	20.05 \pm 1.21
"	<i>hh</i>	28	177.9 \pm 3.75	29.46 \pm 2.65	16.43 \pm 1.48
"	<i>AA</i>	43	186.7 \pm 3.65	35.55 \pm 2.58	19.03 \pm 1.38
"	<i>Aa</i>	64	177.8 \pm 2.86	34.01 \pm 2.02	19.12 \pm 1.14
"	<i>aa</i>	33	172.4 \pm 4.76	40.52 \pm 3.36	23.50 \pm 1.95
Females	<i>CC</i>	27	138.2 \pm 3.25	25.12 \pm 2.30	18.17 \pm 1.66
"	<i>Cc</i>	72	143.9 \pm 1.67	21.00 \pm 1.18	14.58 \pm 0.81
"	<i>cc</i>	34	147.6 \pm 2.24	19.39 \pm 1.58	13.12 \pm 1.06
"	<i>HH</i>	33	145.6 \pm 2.32	20.09 \pm 1.64	13.79 \pm 1.12
"	<i>Hh</i>	67	141.4 \pm 1.87	22.74 \pm 1.32	16.08 \pm 0.93
"	<i>hh</i>	33	145.1 \pm 2.65	22.58 \pm 1.87	15.15 \pm 1.29
"	<i>AA</i>	29	140.4 \pm 2.62	20.78 \pm 1.84	14.80 \pm 1.31
"	<i>Aa</i>	74	144.9 \pm 1.30	23.02 \pm 1.27	15.88 \pm 0.88
"	<i>aa</i>	30	142.7 \pm 2.62	21.60 \pm 1.88	15.13 \pm 1.31

The data of table 5 are based on the weights of seven more rats than the number used in previous tables. The data on the rats which were added at this point were not complete when previous tables were compiled.

genes. In the case of the F_2 males there is an indication that growth genes are carried on the same chromosome with the gene (*C*) for chromogen. Male rats homozygous for this gene are slightly larger than those in a heterozygous condition, and those in a heterozygous condition are larger than pure recessives (those homozygous for the gene *c* for albinism). This condition does not hold true for the females and therefore cannot be true for the males, unless there is a complementary action between growth

TABLE 6

F₂ Males (S₁×S₂) weighing 225 grams or more at 90 days of age.

NUMBER	PHENOTYPE	GENOTYPE	NUMBER IN LITTER	AGE IN DAYS				
				30	50	70	90	150
20c	agouti	<i>Cc Hh Aa</i>	5 (1)	65	135	220	275	340
40a	black	<i>Cc Hh aa</i>	10 (3)	50	100	175	255	320
55a	black hooded	<i>Cc hh aa</i>	6	55	100	170	255	330
21c	agouti	Failed to breed	5 (1)	65	145	210	250	330
1a	p. e. yellow	<i>Cc Hh AA</i>	8 (2)	65	110	175	245	330
10a	p. e. yellow	<i>CC Hh AA</i>	8	65	110	165	240	320
14c	p. e. yellow	<i>CC Hh Aa</i>	10	50	110	180	240	330
4a	albino	<i>cc HH Aa</i>	8 (2)	65	105	170	235	335
39a	agouti	<i>Cc Hh AA</i>	10 (3)	50	100	160	235	300
71a	albino	<i>cc Hh AA</i>	8 (5)	40	110	165	230	300
88b	albino	<i>cc Hh Aa</i>	8	50	120	180	230	270
92b	black	<i>Cc Hh aa</i>	9	50	135	185	230	280
27a	p. e. yellow	<i>Cc HH Aa</i>	8 (4)	65	120	175	225	280
28a	p. e. cream	<i>CC Hh aa</i>	8 (4)	65	115	170	225	245
73a	agouti	<i>Cc Hh Aa</i>	8 (5)	40	100	160	225	280
11c	agouti	<i>CC Hh AA</i>	10 (6)	40	105	175	225	290
12c	agouti	<i>CC HH AA</i>	10 (6)	50	125	180	225	300

F₂ Males (S₁×S₂) weighing 135 grams or less at 90 days of age.

80a	black	<i>Cc HH AA</i>	8 (1)	30	60	100	135	240
81a	p. e. yellow	<i>CC HH Aa</i>	8 (1)	30	60	100	130	195
82a	p. e. yellow	<i>CC HH AA</i>	8 (1)	30	55	100	130	230
90a	albino	<i>cc hh aa</i>	9	35	65	95	130	220
56b	albino	<i>cc hh aa</i>	8	30	55	85	130	205
50c	albino	<i>cc Hh Aa</i>	7	45	70	110	130	190
70c	agouti	<i>Cc HH Aa</i>	8 (2)	40	80	110	130	175
12b	albino	<i>cc Hh Aa</i>	8	35	50	80	125	200
86c	albino	<i>cc Hh Aa</i>	9	20	45	75	125	180
4b	agouti	<i>Cc Hh Aa</i>	10 (3)	35	50	90	120	180
27b	agouti	<i>Cc HH Aa</i>	11	25	45	70	120	200
71c	agouti	<i>CC HH Aa</i>	8 (2)	25	60	110	120	180
8b	p. e. yellow	<i>Cc Hh AA</i>	10 (3)	30	40	80	120	200
3b	agouti	<i>Cc Hh Aa</i>	10 (3)	30	50	85	115	190
62b	black	<i>Cc Hh aa</i>	9	20	55	70	115	175
10b	albino	<i>cc HH AA</i>	10 (3)	30	40	70	110	180
79a	albino	<i>cc Hh aa</i>	8 (1)	30	45	75	105	210
1b	agouti	<i>Cc HH Aa</i>	10 (3)	30	50	70	100	195
41b	albino	<i>cc HH aa</i>	7	20	40	65	100	190

TABLE 7

F₂ Females (S₁×S₃) weighing 170 grams or more at 90 days of age.

NUMBER	PHENOTYPE	GENOTYPE	NUMBER IN LITTER	AGE IN DAYS				
				30	50	70	90	180
58a	p. e. yellow	<i>CC HH Aa</i>	6 (1)	60	110	160	195	230
16a	agouti	<i>Cc Hh Aa</i>	8 (3)	60	105	155	190	190
72b	agouti	<i>Cc Hh Aa</i>	8	30	90	125	180	230
42a	albino	<i>cc Hh Aa</i>	10 (2)	45	85	140	180	210
8a	albino	<i>cc HH Aa</i>	8	55	100	155	180	220
83b	agouti hooded	<i>Cc hh Aa</i>	5	65	130	160	180	205
57a	albino	<i>cc hh aa</i>	6 (1)	50	105	150	175	215
43a	albino	<i>cc hh aa</i>	10 (2)	50	90	135	175	210
66a	agouti	<i>Cc Hh Aa</i>	10	45	95	140	170	200
8c	agouti hooded	<i>Cc hh Aa</i>	8	50	100	145	170	195
51a	albino	<i>cc Hh Aa</i>	9	45	90	130	170	200
14a	black	<i>Cc Hh aa</i>	8 (3)	60	100	135	170	210
60a	agouti	<i>Cc Hh AA</i>	6 (1)	55	100	140	170	220
89b	agouti hooded	<i>Cc hh AA</i>	8	50	105	150	170	200

F₂ Females (S₁×S₃) weighing 115 grams or less at 90 days of age.

51b	agouti hooded	<i>Cc hh Aa</i>	10	30	60	90	115	170
44c	p. e. yellow	<i>CC HH Aa</i>	6	40	75	100	115	140
92a	black	<i>Cc Hh aa</i>	9 (1)	30	60	95	115	200
5b	black	<i>Cc HH aa</i>	10 (2)	35	55	95	115	135
7b	agouti	<i>Cc Hh AA</i>	10 (2)	35	55	100	115	130
43b	p. e. yellow hooded	<i>CC hh AA</i>	7	20	50	80	110	160
16b	agouti	<i>Cc Hh Aa</i>	8 (3)	30	50	75	110	160
15b	agouti	<i>Cc Hh Aa</i>	8 (3)	40	55	80	110	140
95a	albino	<i>cc Hh aa</i>	9 (1)	30	50	80	110	160
93a	agouti	<i>Cc Hh AA</i>	9 (1)	30	60	85	110	155
18b	p. e. hooded yellow	<i>CC hh Aa</i>	8 (3)	40	60	80	110	150
77b	p. e. hooded yellow	<i>CC hh Aa</i>	8	25	50	90	110	140
79c	agouti	<i>Cc hh Aa</i>	9 (5)	25	50	75	105	105
86a	albino	<i>cc HH aa</i>	8 (4)	25	50	70	100	190
85a	p. e. yellow	<i>CC Hh Aa</i>	8 (4)	30	50	75	100	165
14b	agouti	<i>Cc Hh AA</i>	8 (3)	40	60	80	100	140
81c	p. e. hooded yellow	<i>Cc hh Aa</i>	9 (5)	25	45	70	100	145
83a	black	<i>CC Hh aa</i>	8 (4)	30	45	70	90	160
84a	agouti	<i>CC Hh AA</i>	8 (4)	30	40	60	85	150

genes linked with the gene for chromogen and genes carried on the Y chromosome. The same condition exists in the case of males of the agouti (*A*) and non-agouti (*a*) gene group, but again the females of the same gene grouping fail to show any indication of growth genes being carried on the same chromosome with the agouti gene.

Grouping of the Heavy Weight and Light Weight rats

The data of table 5 do not throw any definite light upon the cause of the greater variability in weight of F_2 rats when compared to F_1 rats, and it will be of interest to group the heavy and light F_2 rats of each sex and study their genetic constitution. Tables 6 and 7 contain such groups and in connection with this grouping complete data on each rat are given.

At this point it might be well to indicate the significance of the small letters following the number of each rat and also the numbers in parenthesis following the number of rats in the litter to which these rats belong. The system used in ear marking the rats did not extend beyond ninety-nine; therefore, the numbers of the first ninety-nine F_2 rats are followed by a small "a," the second ninety-nine by a small "b" and the third by a small "c" to distinguish whether they belong to the first, second or third group. The numbers in parenthesis indicate that more than one rat in the male or female division of the table belongs to the same litter, and this same number is placed after each male or female coming from one litter.

Table 6 includes the F_2 male rats weighing 225 grams or more at 90 days of age and the F_2 male rats weighing 135 grams or less at 90 days of age. This table shows that among 141 male rats there were 17 falling in the heavy group and 19 falling in the light group. The 17 heavy weight rats came from 11 different litters and the size of the litters from which they came varied from five to ten young per litter. The 19 light weight rats came from 11 different litters and the size of the litters from which they came varied from six to ten young per litter.

The weights of nine rats (numbers 1b, 3b, 4b, 8b, 10b, 79a, 80a, 81a, and 82a) in the light group can be explained on a nutritional basis and should not be considered from the standpoint of heredity. Rats bearing numbers 1b, 3b, 4b, 8b, and 10b are litter mates from a litter of ten in number. This litter was marked at weaning time as being in an unthrifty condition. This unthrifty condition was indicated by rough coats and lack of vitality. There were six male rats and four females in the litter. One male died when fifty-five days of age and the remaining five males all fall into the light group of males. Two of the four females of this litter

(numbers 5b and 7b) are to be found in the light group of females, table 7. Rats bearing numbers 79a, 80a, 81a, and 82a are litter mates and from a litter of eight in number. This litter was noted at weaning time as being in the same physical condition as the litter previously discussed. There were four males and four females in this litter. All males are found in the light group and all their litter sisters (numbered 83a, 84a, 85a, and 86a) are found in the light group of females (table 7). These are the only rats included in tables 6 and 7 of which a poor physical condition was observed during the time they were on the experiment. By eliminating the nine rats of these two litters there remain ten male rats in the light weight group and these ten rats represent nine different litters.

In studying the remaining rats of these two groups, there is no evidence of any linkage between the known genes and growth genes. Their known genetic combinations are as varied as could be expected in so small a number. They come from a relatively large number of litters and from litters of large size as well as from litters of medium size. There is no evidence that the rats of the heavy group are from smaller sized litters than are the rats of the light group. In fact by the elimination of only one of the litters as previously discussed there would not remain a single rat in the light group coming from a litter above nine in number, while there are five rats in the heavy group belonging to three different litters of ten in number. This would indicate that size is probably inherited as a multiple gene condition, and that chromosomes other than those studied carry the major growth genes, if such exist.

The diploid number of chromosomes of the rat (*Rattus Norvegicus*) according to the recent work of PINCUS (1927) is 42. He found an unequal pair in the spermatogonial divisions which he concluded were the sex chromosomes. Since the present study involves only six diploid chromosomes there is still an ample number of chromosomes not included in the study for an assumption that there are size genes carried on the unstudied chromosomes.

Table 7, which has been referred to in my discussion of table 6, includes the F_2 female rats weighing 170 grams or more at 90 days of age and the F_2 female rats weighing 115 grams or less at 90 days of age. This table shows that among 133 female rats there were 14 falling in the heavy weight group and 19 falling in the light weight group. The 14 heavy weight rats came from 10 different litters and the size of the litters from which they came varied from five to ten young per litter. The 19 light weight rats came from 9 different litters and the size of the litters from which they came varied from six to ten per litter. If we eliminate rats

of the light group belonging to the two litters discussed in connection with table 6, there will remain 13 rats in the light weight group. These 13 rats came from seven different litters and the size of the litters from which they came varied from six to ten young per litter.

The same conclusions can be drawn from the data of table 7 as were drawn from the data of table 6. There is no evidence that size genes are carried on the three pairs of chromosomes studied and no evidence that the size of the litters from which the two groups of rats came influenced the

TABLE 8

Variation in litter size and weight and correlation between litter size and weight of 141 F_2 ($S_1 \times S_3$) males at 90 days of age.

WEIGHT IN GRAMS	SIZE OF LITTER										TOTALS
	3	4	5	6	7	8	9	10	11	12	
100-105					1	1		1			3
110-115							1	2			3
120-125						2	1	2	1		6
130-135					1	5	1				7
140-145					1	4	2	3	3		13
150-155		1		3	3	1	4	2			14
160-165		1		1	4	3	1	1			11
170-175					2	2	5	2			11
180-185	1				4	7	1	5			18
190-195					2	6	3	2			13
200-205	1		1			2	6	3			13
210-215			2		1	3	1	2			9
220-225				1		3	2	3			9
230-235						3	1	1			5
240-245						2		1			3
250-255				1				1			2
260-265											
270-275			1								1
TOTALS	2	2	4	6	19	44	29	31	4		141

Mean litter size = 8.30

Standard deviation = 1.54

Coefficient of variation = 18.55 percent

Mean weight = 177.2 grams

Standard deviation = 35.54 grams

Coefficient of variation = 20.05 percent

Coefficient of correlation (litter size and weight) = 0.10 ± 0.05

size of the rats at ninety days of age. The only explanation which we can offer as to the size difference of these two groups of F_2 rats is that size is inherited as a multiple gene condition and that the major growth genes are carried on the unstudied chromosomes.

Correlation Between Litter Size and Weight

Tables 6 and 7 indicate that size of litter has little if any effect upon the weight of F_2 ($S_1 \times S_2$) rats. In order to know the definite relationship between litter size and weight, correlation tables have been made for both male and female rats. Tables 8 and 9 show that a very slight correlation,

TABLE 9

Variation in litter size and weight and correlation between litter size and weight of 133 F_2 ($S_1 \times S_2$) females at 90 days of age.

WEIGHT IN GRAMS	SIZE OF LITTER									
	3	4	5	6	7	8	9	10	11	TOTALS
80-85						1				1
90-95						1				1
100-105						3	2			5
110-115				1	1	3	4	3		12
120-125				1	3	5	3	4		16
130-135		1			3	5	4	6	5	24
140-145		1	2	2	3	6	3	2	2	21
150-155			1	1	1	6	7	5	1	22
160-165					3	7	3	3		16
170-175			1	2		3	1	2		9
180-185			1			2		1		4
190-195				1		1				2
TOTALS:		2	5	8	14	43	27	26	8	133

Mean litter size = 8.38

Standard deviation = 1.52

Coefficient of variation = 18.13 percent

Mean weight = 141.30 grams

Standard deviation = 21.52 grams

Coefficient of variation = 15.23 percent

Coefficient of correlation (litter size and weight) = 0.12 ± 0.06

but of doubtful significance, exists between the litter size and weight of F_2 rats at 90 days of age, the coefficient of correlation being 0.10 ± 0.05 for the male rats and 0.12 ± 0.06 for the females. These coefficients of correlations indicate that we are safe in saying that the size of the litter had very little effect upon the weights of the rats of the F_2 generation. These tables include the two litters which were eliminated from tables 6 and 7 on the ground that their small size could not be due to heredity.

Various Gene Combinations

Table 10 gives the average growth weights of F_2 ($S_1 \times S_3$) rats of various gene combinations. This table included all tested F_2 rats, or 141 males and 133 females. The rats were first grouped according to the number of

dominant genes and then according to the homozygous or heterozygous condition of the genes. After a careful study of the data of this table,

TABLE 10

Average weights of $F_2(S_1 \times S_3)$ animals of various gene combinations at ages 30-150 days.

SEX	GENE COMBINATION	NUMBER OF INDIVIDUALS	AGE IN DAYS				
			30	50	70	90	150
Males	3 Dominant	67	42.98	88.95	134.32	180.37	266.41
"	2 "	55	40.90	85.72	131.45	175.09	241.27
"	1 "	15	44.33	84.33	128.33	176.66	242.00
"	0 "	4	32.50	68.75	108.75	150.00	222.50
Females	3 "	59	40.00	80.33	141.61	139.70	176.77
"	2 "	55	40.54	78.81	112.00	139.40	177.72
"	1 "	15	40.33	82.66	113.00	141.66	182.33
"	0 "	4	43.75	91.25	135.00	161.25	197.50
Males	3 Homozygous	26	39.61	80.38	117.69	165.60	232.69
"	2 "	56	43.05	89.28	134.10	179.00	248.30
"	1 "	47	42.97	88.29	135.51	179.20	243.51
"	3 Heterozygous	12	38.75	81.25	132.08	180.40	241.66
Females	3 Homozygous	16	39.06	82.18	117.50	142.80	183.75
"	2 "	46	42.93	82.60	114.67	139.90	180.65
"	1 "	46	40.10	78.15	109.13	140.10	179.02
"	3 Heterozygous	25	38.20	78.80	110.80	140.20	176.80

I am convinced that there is no relation between the size of rats and the number of known dominant genes, or between size and the homozygous and the heterozygous condition of the genes studied.

CONCLUSIONS ON EXPERIMENTAL WORK

In this study of three known pairs of allelomorphic genes there is no evidence that the known dominant genes, or that the heterozygous condition of the genes studied has any influence on the size of F_2 rats of the S_1 and S_3 cross.

There is no definite evidence that growth genes are linked with the genes studied, but on the other hand there is no evidence that growth genes are not carried on chromosomes not studied. The rats of the F_2 generation of the S_1 by S_3 cross were more variable than the F_1 rats of this cross, and when the heavy and light rats of the F_2 generation are grouped for study there is evidence that the difference in size of the rats of these two groups

must be due to hereditary size genes probably carried on the unstudied chromosomes.

This study does not give an answer to the question as to the cause of hybrid vigor or heterosis, but it does give additional information on the three pairs of chromosomes studied.

SUMMARY OF EXPERIMENTAL RESULTS

1. Hybrid vigor or heterosis was shown by the F_1 rats in each cross studied.
2. Heterosis was more marked in the F_1 rats of the S_1 by S_2 cross than in the F_1 rats of the S_1 by S_3 cross, as would be expected since there was a greater difference in the size of the parent races in the former cross.
3. There was a distinct loss of vigor shown by the F_2 rats of each cross as compared to the F_1 rats.
4. This loss of vigor was more marked in the case of the F_2 rats of the S_1 by S_3 cross.
5. The F_1 rats of each cross were less variable in weight than the rats of the respective parent strains.
6. The F_2 rats of each cross were more variable in weight than F_1 rats, although the difference in the variability was not sufficient to be significant in the case of the F_2 rats of the S_1 by S_2 cross.
7. The F_2 rats of each cross were less variable than the rats of the respective parent strains.
8. The grouping of F_2 rats of the S_1 by S_3 cross according to known allelomorphous genes does not show any definite evidence that the three pair of chromosomes studied carry growth genes.
9. The grouping of the heavy weight and light weight F_2 rats does not show any relation between the size of the rats and their known genetic constitution. This grouping also fails to indicate that size of the litters, from which the heavy weight and light weight rats came, has influenced the weight of the F_2 rats.
10. There exists a very slight correlation between the litter size and the weight of F_2 rats, but this correlation is too small to account for the extreme difference in the weights of rats contained in the heavy weight and the light weight groups of tables 6 and 7.
11. The grouping of F_2 rats according to the number of known dominant genes in their genetic constitution does not show any evidence that these genes influence the size of the rats.
12. The grouping of F_2 rats according to the homozygous or heterozygous condition of the known allelomorphous genes does not show any evi-

dence that either the homozygous or heterozygous state has any influence on the size of the rats.

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THE CYTOLOGY AND GENETICS OF A HAPLOID SPORT FROM *OENOTHERA FRANCISCANA*¹

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The first haploid spermatophyte to be recorded was from *Datura stramonium* (BLAKESLEE and others 1922), and later studies have shown them to be not infrequent sports from this species apparently appearing without marked relation to the temperature of the season. They have also been obtained by the application of pollen from *Datura ferox* to the stigma of *D. stramonium*. BELLING and BLAKESLEE (1923, 1927) presented a cytological study of these plants. Shortly after, CLAUSEN and MANN (1924) described two haploid plants from the F₁ of the cross *Nicotiana tabacum* × *N. sylvestris*, and later CHIPMAN and GOODSPEED (1927) gave an account of the haploid from *Nicotiana tabacum* var. *purpurea*. RUTTLE (1928) reported three additional haploids from *tabacum* var. *purpurea* and two new plants from *tabacum* var. *Cuba*. GAINES and AASE (1927) in attempt to pollinate a hybrid of Triticum from Aegilops obtained a haploid wheat plant and described its cytology. LESLEY and FROST (1928) found a dwarf plant of *Mattiola* having the haploid set of normal chromosomes in addition a chromosome fragment. HOLLINGSHEAD (1928) recorded haploids of *Crepis capillaris* from the F₁ of the cross *capillaris* × *tech*. JÖRGENSEN (1928) has obtained a number of haploids of *Solanum nigrum* and *S. nigrum gracile* through capsules stimulated to parthenocarpic development by pollinations from *S. luteum* and other forms that hybridize only rarely with *S. nigrum*. JÖRGENSEN gives an account of the cytology of these *Solanum* haploids in which the behavior at meiosis is very different from that in Triticum, *Nicotiana*, *Datura* and as we shall see in *Oenothera*. LINDSTROM (1929) described a haploid from tomato almost completely pollen sterile but producing a few seeds after pollinations from other varieties. Our paper adds to the list the first haploid *Oenothera* but it seems probable that other plants of this character will shortly be recognized in this genus.

The possibility of apomixis in *Oenothera* has been strongly indicated by the interesting results of HABERLANDT (1921, 1922, 1927) in connection

¹ Cytological Studies on *Oenothera* IV. Papers from the Department of Botany, UNIVERSITY OF MICHIGAN, No. 303.

with his experiments on the stimulus to growth resulting from wounds. By pinching the ovaries, by pricking them with a needle, and by the castration of young flowers he obtained early stages of parthenogenetic embryos and adventitious embryos in *Oenothera Lamarckiana*, together with endosperm development. HABERLANDT holds that the exciting agents are hormones liberated as the result of the wounding of tissue or the death of cells. Similar developments were noted in ovaries of *Lamarckiana* and *muricata* at the end of the flowering season and are believed by him to result from products formed by the aging plants.

There have been other suggestions of possible apomixis in *Oenothera*. LUTZ (1909) reported two plants of *lata* (15 chromosomes) in the F_1 of the cross *lata* \times *gigas*. GATES (1909, 1924) performed experiments on *Lamarckiana-lata*, *biennis-lata* and *gigas* but obtained no seed. The brief statement of HAIG-THOMAS (1913) of parthenogenesis in *Oenothera biennis* following the castration of young flower buds can scarcely be accepted as demonstration. It may happen that parthenogenesis in *Oenothera* will result, as JÖRGENSEN (1928) found in *Solanum*, through pollinations from males that will induce parthenocarpy or through a very limited amount of self pollination sufficient to start the development of fruit and with this will give physiological conditions favorable to forms of apomixis. The known reactions of many plants as expressed in parthenocarpy show a physiological relation between the development of fruit and stimuli of pollen tube growth. The maturation of seed in many forms is apparently only possible as it proceeds hand in hand with the development of the fruit.

GENETICS OF THE HAPLOID

Oenothera franciscana Bartlett, text figure 1, is one of the few apparently homozygous forms in this genus of numerous impure or heterozygous species. It is a large vigorous plant with pollen almost wholly perfect, text figure 3A, and with high seed fertility (about 90 percent). As would be expected its chromosomes are characteristically paired at diakinesis and during the first meiotic division. In certain material (CLELAND 1922) a chain of four chromosomes has been found persisting even to metaphase of the heterotypic mitosis but in other material (Kulkarni 1929) this chain breaks into two pairs at diakinesis showing the association to be loose in character. For eight years cultures with a total of 1499 plants gave no exceptions to the type. Then in 1923 appeared the first variant, a sport named *pointed tips* which later was found to be a haploid, presumably from the parthenogenetic development of a *franciscana* egg.

Plants of pointed tips, text-figure 2, are about half the stature of *franciscana* and with all organs proportionally smaller. The leaves are generally narrower and more sharply pointed and the very red bud cones are more attenuate. The flowers are about half the size of the parent species. Pollen is developed only in small amounts and frequently flowers will produce none at all, the anthers being shriveled. Some plants have

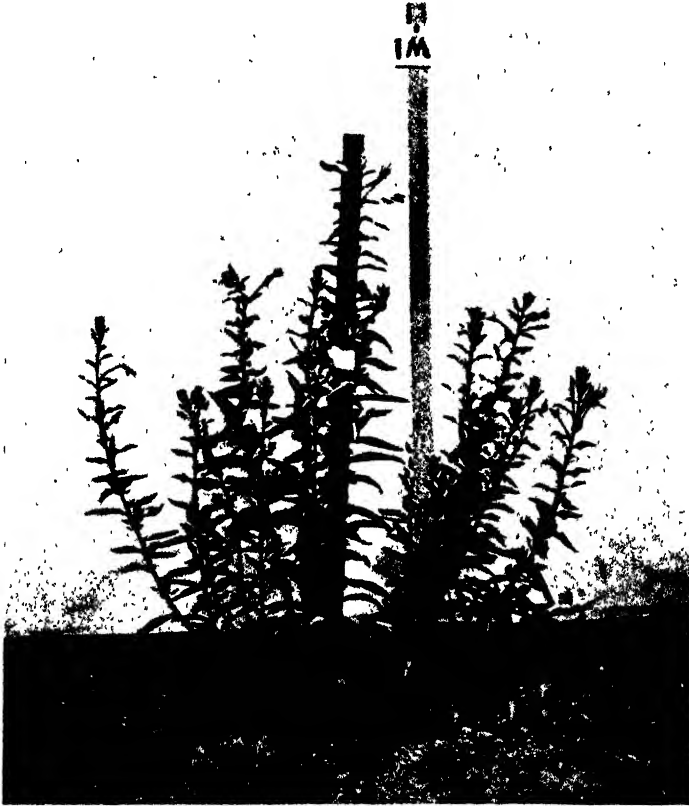


FIGURE 1.—*Oenothera franciscana* early in the flowering season. The central shoot may finally become more than 1 m. high.

been found to develop no pollen over several weeks of daily observation. In such pollen as may be formed, text figure 3B, frequently 60 to 90 percent of the grains will be shrunk. Since the pollen is so highly sterile few seeds are developed on selfed plants and the capsules are thin.

The first plant of pointed tips (23.21-165) was selfed and also back-crossed to the parent line of *franciscana*. The progeny of selfed lines is

almost wholly plants of the parent type, *franciscana*, but a few plants of pointed tips appear. The data of the selfed lines are given in table 1 and it should be noted that cultures 26.25 and 26.26 are second generations and that cultures 27.41, 27.42 and 27.43 are third generations. These selfed lines gave a total of 694 *franciscana* and 29 pointed tips. The behavior of the haploid when selfed was then what would be expected. Its fertile gametes carry the haploid set of *franciscana* chromosomes and their



FIGURE 2.—The haploid, pointed tips, a plant of about one half the stature of *Oenothera franciscana*.

fusion would give the diploid zygote of *franciscana*. Such plants of pointed tips as appear are to be considered as coming from eggs that develop parthenogenetically. Of the pointed tips, 22 plants in one line (26.26 and 27.43) presented somewhat broader leaves than the type and a higher degree of pollen fertility; this form has not yet been studied cytologically and may possibly be a modification of the original haploid. A very interesting form, *red elongate*, which appeared in certain cultures, table I, will not be discussed at this time.

In addition to the first plant of pointed tips (23.21-165) there have appeared from later cultures of *franciscana* 3 more plants:—27.21-1108 from a culture numbering 1117 plants, 28.21-1 from a culture of 945 plants, and 28.61-813 from a culture of 847 plants. The total number of haploids so far thrown by *franciscana* has been 4 plants in thirteen genera-

TABLE 1
Progeny in selfed lines from the first plant of pointed tips (23.21-165).

CULTURE	PARENT POINTED TIPS SELFED	PERCENT OF GER- MINATION	SEED- LINGS	FRAN- CIS- CANA	POINTED TIPS	RED ELON- GATE	FAILED TO MATURE	DIED
24.25 1st generation	23.21-165	94	361	329	24.25-248 24.25-332		1 dwarf similar to pointed tips	29
26.25 2nd generation	24.25-248	74	37	29	26.25-13 26.25-14 26.25-20			5
26.26 2nd generation	24.25-332	68.2	60	55	26.26-40 somewhat broader leaved		2 narrow- leaved rosettes	2
27.41 3d generation	26.25-13	75	138	107	27.41-82 27.41-130	13	1 large thick- leaved rosette 8 narrow- leaved rosettes	7
27.42 3d generation	26.25-14	25.7	51	39		3	1 narrow- leaved rosette	8
27.43 3d generation	26.26-40	53.4	164	135	21 plants somewhat broader leaved	1	1 large thick- leaved rosette	6
Total			811	694	29	17	14	57

tions that have totaled 5690 plants. However, some of these generations have been small and did not express the results of complete seed germination so that the ratio of pointed tips out of *franciscana* is undoubtedly higher than these figures might suggest. Since the cultures 27.21, 28.21, and 28.61 were from seeds forced to complete germination and gave 3

pointed tips in a total of 2909 plants, it seems probable that the ratio of the haploid to *franciscana* is about 1:1000.

Pointed tips has also appeared in some crosses of *franciscana* to certain derivatives upon which the senior author is making studies on the inheritance of the *sulfurea* color and of dwarfness. The following three crosses threw each a single plant of the haploid:—culture 26.29 *franciscana* × *franciscana sulfurea*, 49 plants, gave pointed tips 26.29-6; culture 26.58 *franciscana* × (*franciscana* × *franciscana sulfurea* dwarf), 254 plants, gave pointed tips 26.58-103; culture 26.61 (*franciscana sulfurea* dwarf × *franciscana*) × *franciscana*, 214 plants, gave pointed tips 26.61-84. It is clear that pointed tips in cultures 26.29 and 26.58 might have come from the parthenogenetic development of *franciscana* eggs. Culture 26.61 must be briefly explained: its female parent was the hybrid *franciscana sulfurea* dwarf (*yytt*) × *franciscana* (*YYTT*); *Y* stands for yellow petals and *T* for normal height, absence of color (*sulfurea*) and dwarfness being recessive. The hybrid has the constitution *YyTt* and forms gametes *YT*, *Yt*, *yT* and *yt*. The egg *YT* has the chromosome set of *franciscana* and its parthenogenetic development would give pointed tips. The progeny of the three plants of pointed tips is given in table 2. As would be

TABLE 2

Progeny from pointed tips out of crosses involving *franciscana* (*f*), *franciscana sulfurea* (*fs*), and *franciscana sulfurea dwarf* (*fsd*).

CULTURE	PARENT POINTED TIPS SELFED	PARENT CROSS	PERCENT OF GER- MINATION	SEED- LINGS	FRAN- CIS- CANA	POINTED TIPS	OTHER FORMS	FAILED TO MATURE	DIED
27.44	26.29-6	<i>f</i> × <i>fs</i>	28.7	277	249	none	8 broad-leaved dwarfs 3 thick, broad leaves	6 weak rosettes	11
27.45	26.58-103	<i>f</i> × <i>fsd</i>	39.2	126	115	27.45-112	2 light green 1 thick, broad- leaved rosette	3 weak rosettes	4
27.46	26.61-84	(<i>fsd</i> × <i>f</i>) × <i>f</i>	45.1	60	53	none	1 broad-leaved dwarf	1 weak rosette 1 narrow- leaved dwarf	4
Total				463	417	1	15	11	19

expected almost all of the plants were *franciscana*. Only one pointed tips appeared, but there were several exceptional plants the most interesting of which were *light green*, a new type in the lineage of *franciscana* and certain large, thick-leaved forms not yet fully studied.

The progeny of the back-crosses of pointed tips to the parent *franciscana*, table 3, was a total of 531 plants of *franciscana* and 3 dwarfs that failed to mature; there were no pointed tips. The *franciscana* plants were to be expected since the haploid gametes of pointed tips when formed

TABLE 3
Backcrosses of the first plant of pointed tips to the parent species franciscana.

CULTURE	BACKCROSS	PERCENT OF GERMINATION	SEED- LINGS	FRANCIS- CANA	FAILED TO MATURE	DIED
24.26	franciscana \times pointed tips 23.21-169 \times 23.21-165	78.9	244	234	2 dwarfs	8
24.27	pointed tips \times franciscana 23.21-165 \times 23.21-169	83.7	298	297	1 dwarf	
Total			542	531	3	8

have the same set of chromosomes as that carried by *franciscana* and their union with *franciscana* gametes would give the latter plant. If plants of pointed tips had appeared they would be interpreted as the result of parthenogenetic development of eggs whether from *franciscana* or from pointed tips.

It is appropriate to this paper to record the appearance of pointed tips in a large culture of the so-called *Oenothera Hookeri* of genetical literature. This plant is believed by BARTLETT not to be *Oenothera Hookeri* Torrey and Gray, the type specimen of which has marked canescent pubescence and is perhaps related to *Oenothera venusta* Bartlett. However, for the present it seems best to use the name *Hookeri* for the material first studied by DE VRIES and later by RENNER and others. The material of *Hookeri* is very closely related to *Oenothera franciscana* Bartlett and for purposes of field study might be considered a form of this species. *Hookeri* is not so sturdy a plant but it has the habit, pubescence and bud tips of *franciscana*, differing chiefly in its narrower leaves, bright red stems, red hypanthia, and in having more red on the bud cones. As in *franciscana* the pollen of *Hookeri* is almost wholly perfect and the seed fertility is very high (about 96 percent). *Hookeri* like *franciscana* has pairing chromosomes and is one of the few established homozygous species of *Oenothera*.

The line of *Hookeri* came from seeds kindly supplied by RENNER. Three generations have been grown:—culture 24.18 matured 202 plants, uniform; culture 27.26 matured 526 plants, uniform except for the plant of pointed tips (27.26-475); cultures 28.26 matured 563 plants, uniform.

Pointed tips from *Hookeri* has then appeared only once in a line of cultures totaling 1291 plants, a frequency similar to that in *franciscana* where the ratio has been about 1:1000. This plant (27.26-475) was typical pointed tips but wholly sterile as observed for 38 days during the flowering season. The flowers over this period produced only shriveled anthers.

In concluding the genetical account of this haploid *Oenothera* it should be emphasized that in every case the haploid might have arisen through the parthenogenetic development of an egg thus supporting the evidence so far reported for this manner of origin of haploids in plants.

CYTOLOGY OF THE HAPLOID

The material for this cytological study came from the anthers of pointed tips 27.21-1108, the second plant of pointed tips to appear in the direct line of *franciscana*. The best results were from material fixed in the following modifications of Bouin's fluid: Sat. aq. sol. of picric acid 75 cc, commercial formalin 25 cc, glacial acetic acid 5 cc, chromic acid 1 g, urea 2 g. The anthers were left 5 1/2 hours in the fixing fluid, then rinsed in water and run through low grades of alcohol beginning with 2 1/2 percent, thirty minutes in each grade. Strong Flemming also gave satisfactory fixation; weak Flemming proved worthless. Sections were cut about 10 μ and stained with iron alum haematoxylin.

The haploid count of 7 chromosomes was obtained in tissues of the developing flower. It is shown in plate 1, figure 1, which is the metaphase of a tapetal cell viewed from the pole.

There will first be described the history, illustrated in figures 2-29, which results in the formation of the very few good pollen grains that carry the normal set of 7 chromosomes. The resting nucleus of the microsporocyte, figure 2, contains a delicate chromatic network with numerous deeply staining bodies distributed over the periphery. As prophase comes on, figure 3, there takes place a gradual shortening and condensation of these strands which later, figure 4, give a coarse reticulum with threads of uneven thickness. The threads of this haploid reticulum are distinctly wider than the threads of the diploid *franciscana* at corresponding stages as shown in the excellent figures of CLELAND (1922, figures 1-4).

The stage of synizesis is clearly marked. It begins with the usual apparent contraction of the reticulum, figure 5, the threads showing irregular thickening. The point of greatest condensation with the contracted reticulum against the flattened nucleolus, figure 6, gives a picture similar to that of *franciscana* except that the threads are not so delicate and the

thread system seems to be not so long (see CLELAND 1922, figures 5-8). The emergence from synizesis, figure 7, through the apparent expansion of loops also follows the usual history in *Oenotherae*.

Out of synizesis comes the stage of the open spireme, figures 8 and 9, with the thickened threads rather evenly distributed through the nucleus. It becomes evident that the thread system is not so extensive as that in the diploid nucleus of *franciscana* at the corresponding stage. This is shown by a comparison of figures 8 and 9 with figures 14 and 15 given by CLELAND (1922) for *franciscana*.

The stage of second contraction is as clearly marked as in *franciscana*. The chromatic threads thicken and gather towards the center of the nucleus, figure 10. At this time it is evident that regions of the spireme are to become chromosomes, figure 11, although these structures are very closely grouped. With the loosening of the contracted chromatic mass, figures 12 and 13, the 7 chromosomes of this haploid set appear clearly outlined and the period of the second contraction comes to an end.

Then follows the stage corresponding to the characteristic period of diakinesis in diploid plants, but as would be expected there is here no pairing of chromosomes. The 7 chromosomes become irregularly distributed through the nuclear area as shown in figures 14-18. Occasionally two chromosomes may be found attached end to end by a delicate thread, plate II, figures 14 and 15, which possibly represents a portion of the original thread system still persisting.

The nucleus now makes an attempt to carry through a division at this period of the heterotypic mitosis in diploid plants. A multipolar spindle is shown in figure 19, but this fails to pass into the normal bipolar structure in the material which we are now considering. One pole may develop more or less clearly, figures 20 and 21, but the second pole is not formed, the spindle fibers ending over a broad and vague area. The 7 chromosomes move somewhat towards the single pole and then gather in a group and a nuclear membrane is formed around them, figure 22. This gives a nucleus in the period corresponding to interkinesis of normal meiosis but there has been no segregation of chromosomes, no heterotypic mitosis.

At this time the chromosomes divide lengthwise, and the nucleus then contains 7 split chromosomes, figure 23, such as are present at interkinesis in *franciscana*. As in *franciscana* the split does not appear until rather late in this period that corresponds to interkinesis.

Then comes the homoeotypic mitosis which is to separate the halves of the split chromosomes. Its metaphase, figure 24, with the 7 pairs of daughter chromosomes, now much condensed, is similar to the homoeo-

EXPLANATION OF PLATES

All figures were drawn with the aid of a camera lucida under the Zeiss apochromatic objective 1.5 mm (num. aper. 1.30) in combination with the compensating ocular K 20X. The approximate magnification for figures 1-29 is 2400 diam.; for figures 30-37 it is 1800 diam.

Figures 1-29 give the history which results in the formation of a small proportion of the pollen grains with a normal set of seven chromosomes.

PLATE I

FIGURE 1.—Metaphase of the mitosis in a tapetal cell as seen from the pole of the spindle, 7 chromosomes.

FIGURE 2.—Resting nucleus of the microsporocyte. Many deeply staining chromatic bodies appear near the periphery of the nucleus.

FIGURE 3.—Gradual thickening of the threads, some are still slender.

FIGURE 4.—A later stage in the process of thickening giving a coarse system of uneven threads.

FIGURE 5.—Early synizesis showing the contraction of the thread system.

FIGURE 6.—Mid-synizesis. Most of the threads are in closely twisted coils at the side of the large and rather flattened nucleolus.

FIGURE 7.—Late synizesis. The threads more loose, uniform and thicker.

FIGURE 8.—Open spireme. The threads show bead-like structures, probably chromomeres.

FIGURE 9.—A later stage of the open spireme with threads more uniform in thickness.

FIGURE 10.—Second contraction. Note the central thickening of the threads and the peripheral loops.

FIGURE 11.—Late second contraction. Chromosomes distinct but much crowded.

FIGURE 12.—End of the second contraction. The 7 chromosomes are still clustered around the large nucleolus.

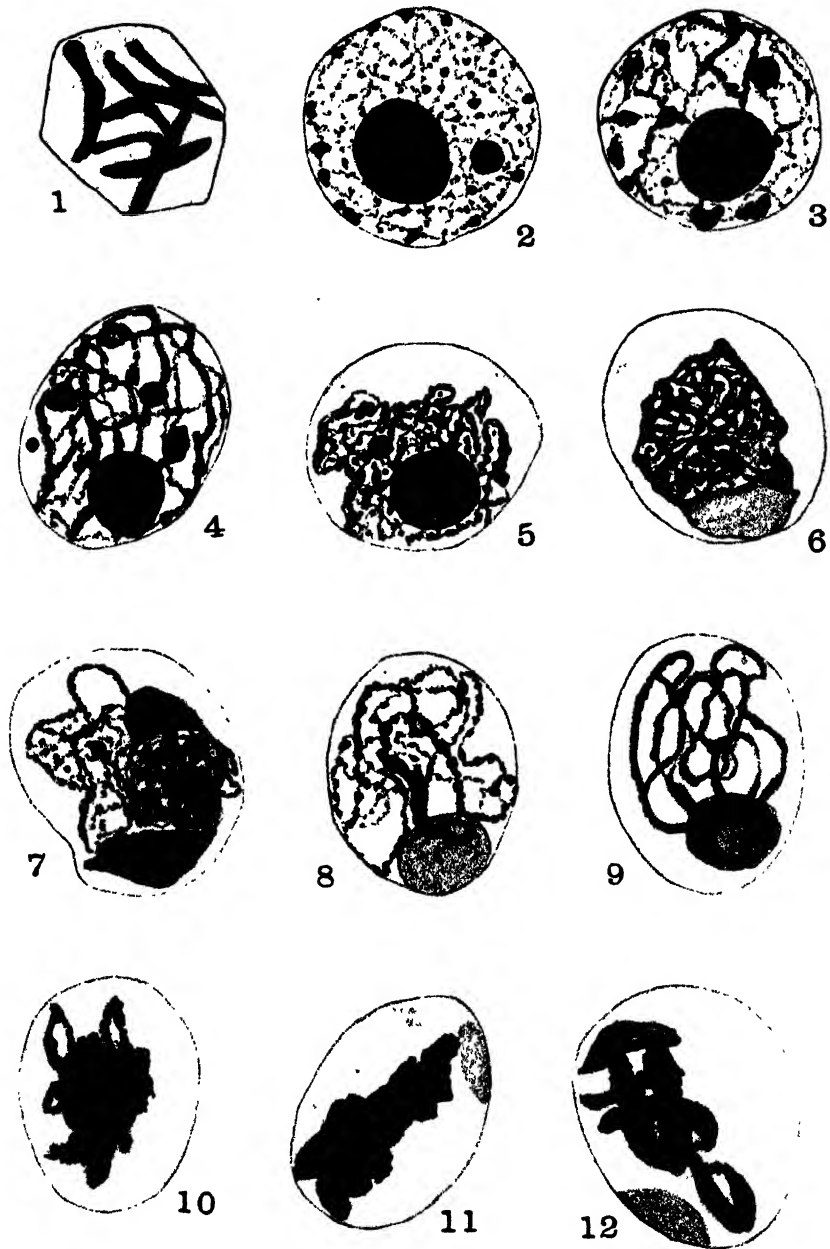


PLATE 2

FIGURE 13.—Chromosomes shortly after their emergence from second contraction.

FIGURES 14, 15, 16, 17, 18.—Late prophase corresponding to diakinesis. The 7 chromosomes distinct.

FIGURE 19.—Multipolar spindle. The 7 chromosomes gathered in the center of the spindle.

FIGURE 20.—Unipolar spindle. The 7 chromosomes in a plate arrangement.

FIGURE 21.—Unipolar spindle. The 7 chromosomes moving somewhat towards the pole.

FIGURE 22.—Reconstituted nucleus following non-reduction, 7 chromosomes.

FIGURE 23.—Reconstituted nucleus in stage corresponding to interkinesis, 7 split chromosomes.

FIGURE 24.—Metaphase of the homoeotypic mitosis, 7 split chromosomes at the equatorial plate.

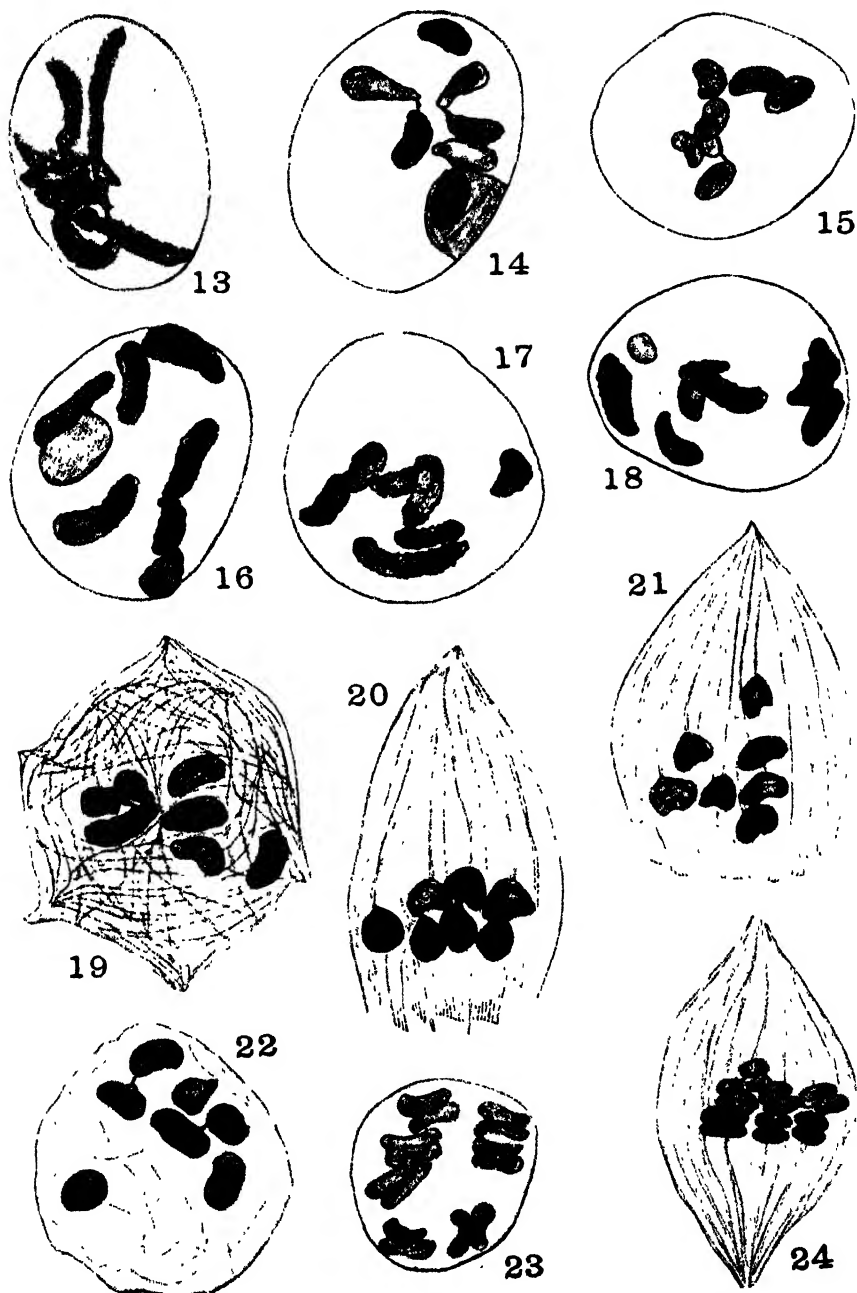


PLATE 3

FIGURE 25.—Homoeotypic anaphase showing two groups of chromosomes, 7 in each group.

FIGURE 26.—Late homoeotypic anaphase, a group of 7 chromosomes viewed from the pole of the spindle.

FIGURE 27.—Telophase of homoeotypic mitosis.

FIGURE 28.—Diad of young pollen cells within the microsporocyte.

FIGURE 29.—Diad of young pollen cells. A mass of cytoplasm has been pinched off and lies at one side.

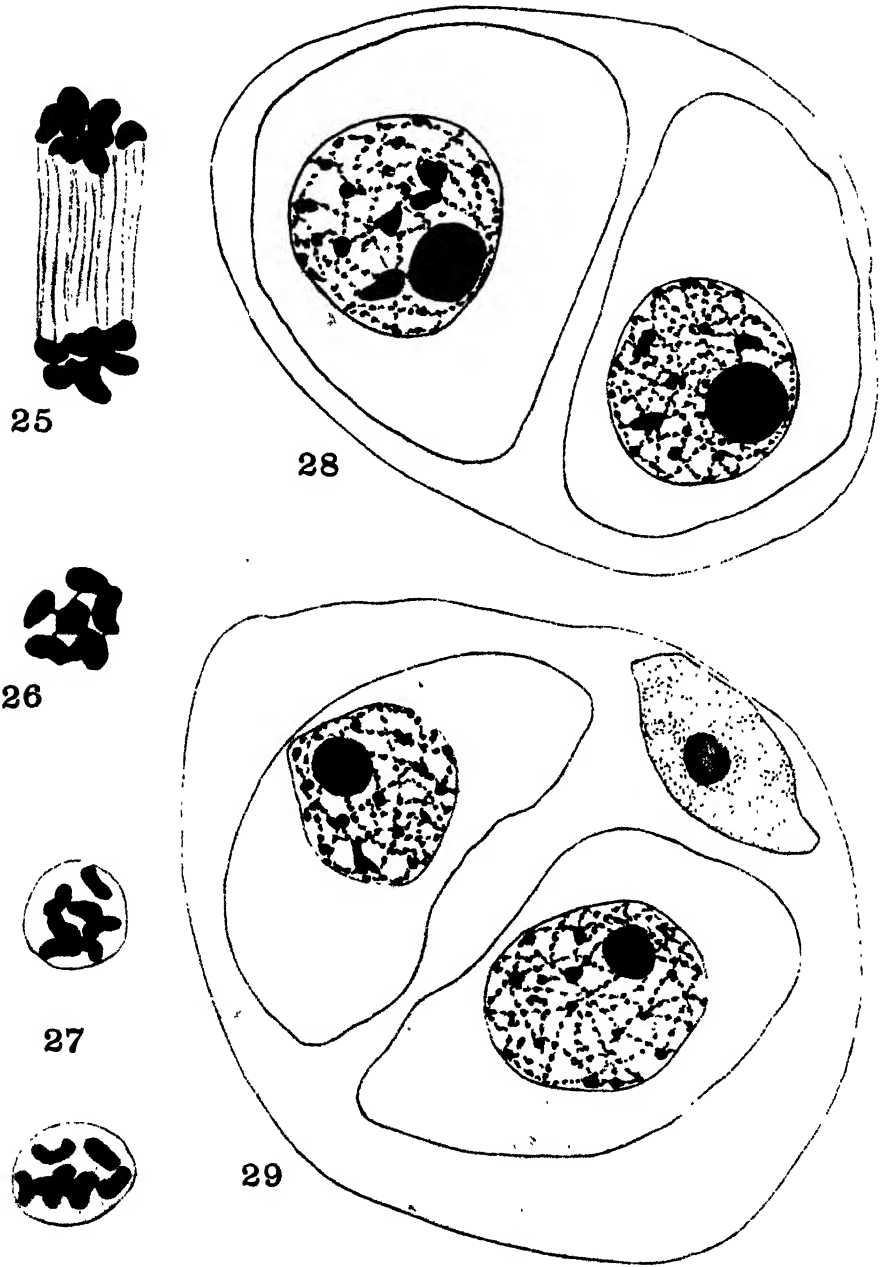


PLATE 4

Figures 30-35 show irregular distribution of chromosomes during meiosis which will result in shrivelled pollen grains.

FIGURE 30.—Heterotypic anaphase; 6 chromosomes passing to one pole and 1 to the other.

FIGURE 31.—Late heterotypic anaphase; 5 chromosomes at one pole and 2 at the other.

FIGURE 32.—Heterotypic anaphase; 5 chromosomes passing to one pole and 2 to the other.

FIGURE 33.—Heterotypic anaphase; 4 chromosomes passing to one pole and 3 to the other.

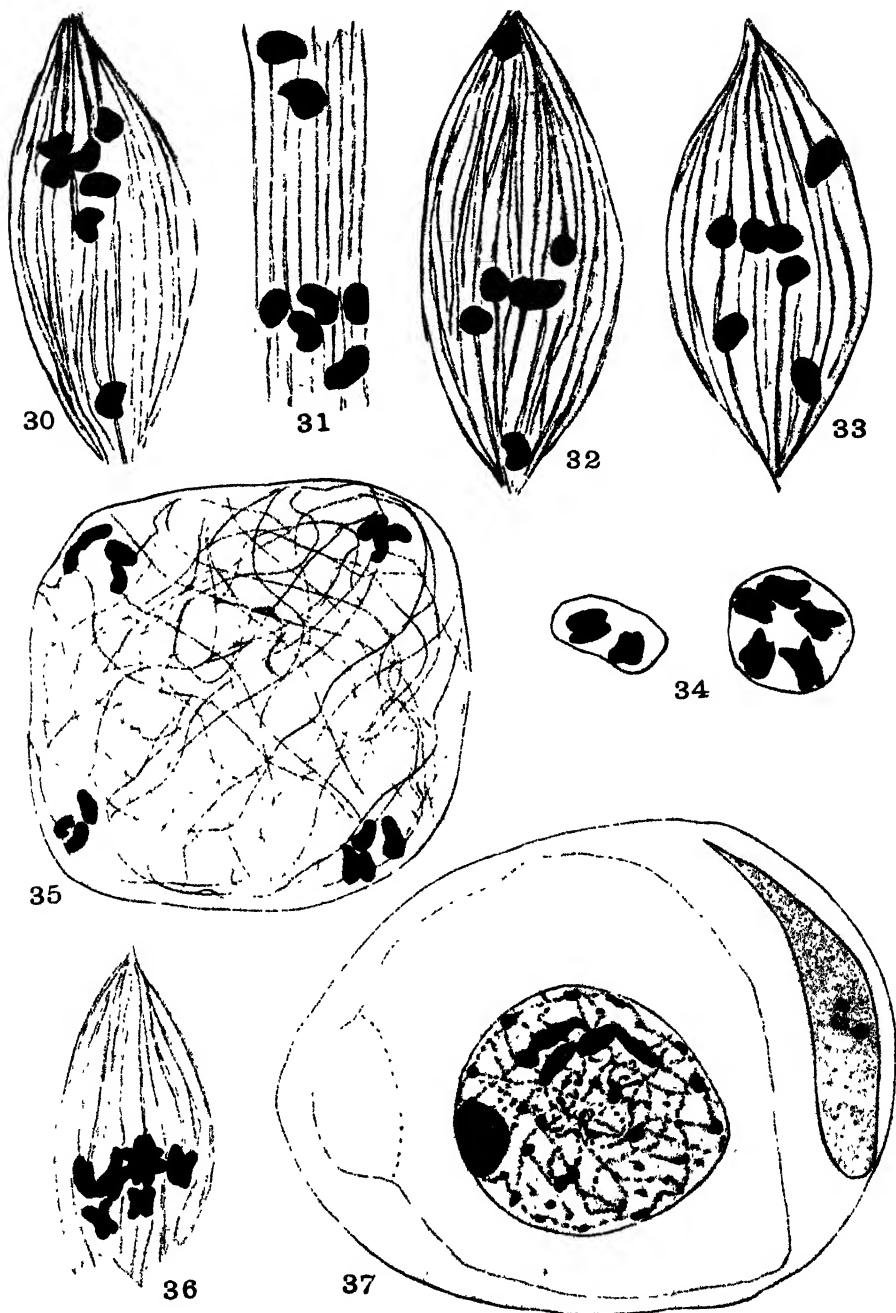
FIGURE 34.—Interkinesis with split chromosomes; five bivalents in one nucleus and two in the other.

FIGURE 35.—Homoeotypic anaphase showing irregular distribution of the 14 chromosomes, two nuclei with 3 and two nuclei with 4 chromosomes.

Figures 36 and 37 show the possible formation of giant pollen grains with 14 chromosomes, a double haploid set.

FIGURE 36.—A unipolar spindle in the homoeotypic mitosis following non-reduction in the heterotypic; 7 split chromosomes, remaining together, will produce a nucleus with 14 chromosomes.

FIGURE 37.—A giant pollen cell accompanied by a mass of pinched off cytoplasm. Such a cell will possibly produce a giant pollen grain with 14 chromosomes.



typic metaphase of *franciscana*, but there is only one spindle in the sporocyte. Anaphase follows, plate 3, figure 25, and the sets of daughter chromosomes move to the poles, figure 26, to organize two daughter nuclei, figure 27, each with 7 chromosomes.

The two nuclei enlarge, pass into a resting condition, the cytoplasm divides, figure 28, and two pollen grains are developed within the microsporocyte. Figure 29 shows the same result with the complication that a small mass of cytoplasm has separated from the two daughter cells. MATSUDA (1928) reports for *Petunia* similar divisions leading to small masses of cytoplasm without nuclei. These pollen grains are haploid and

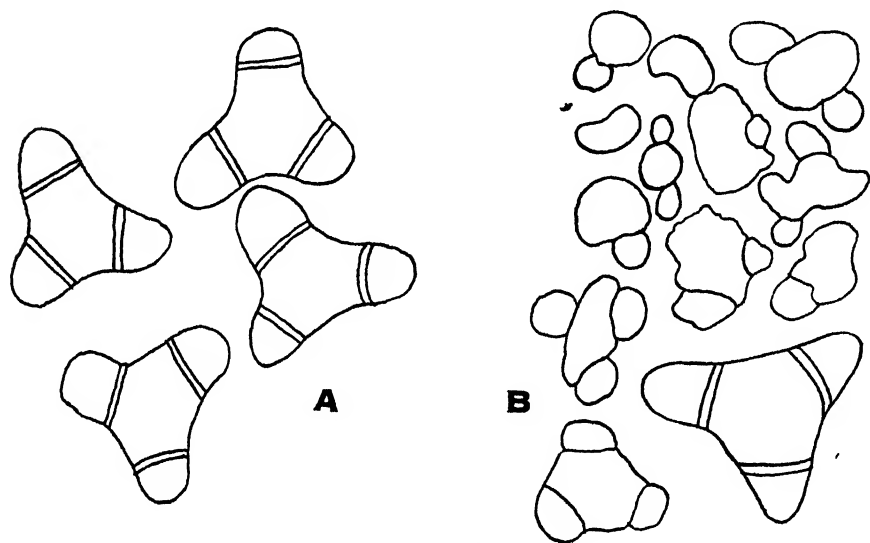


FIGURE 3.—A. Pollen of *Ornothera franciscana*. B. Pollen of the haploid, pointed tips; one good grain is shown among shriveled examples.

they are believed to constitute the fertile pollen which produces *franciscana* zygotes when pointed tips is selfed and when *franciscana* is pollinated by pointed tips. These good pollen grains are of the same size as those of *franciscana*, text figure 3B.

Most of the pollen grains, when produced at all by pointed tips, are shriveled, text figure 3B. Such samplings and estimates as have been made indicate that rarely more than 10 percent of the pollen appears normal in the small amount that an anther may produce. The shriveled grains are to be explained through irregular distribution of chromosomes during meiosis giving grains with less than 7 chromosomes as illustrated

in the series of plate 4 figures, 30–35, which will now be described. In pointed tips there is of course very much more of this material than that which results in pollen grains with the haploid set of 7 chromosomes.

The irregularities of distribution come during the heterotypic mitosis when bipolar spindles are formed and the 7 chromosomes are separated in the possible assortments of 1 and 6, 2 and 5, 3 and 4. All of these possible results of segregation have been observed and they are illustrated in figures 30–33; the attachments of spindle fibers show the direction of chromosome movement. Figure 30 presents the distribution of 1 and 6, figures 31 and 32 the distribution of 2 and 5, and figure 33 the distribution of 3 and 4.

The results of these divisions are microsporocytes containing a larger and a smaller nucleus. Figure 34 illustrates a case following a segregation of chromosomes, 2 and 5. During the interkinesis the chromosomes split lengthwise, figure 34, and the daughter chromosomes are distributed in the usual way by the homoeotypic mitosis, figure 35, which generally ends with the formation of 4 cells that become abortive pollen grains.

Of particular interest in this material of pointed tips was the discovery of behavior which might lead to the formation of giant pollen grains with 14 chromosomes, a double haploid set. In figure 36 is shown a unipolar homoeotypic spindle with its 7 split chromosomes. This condition must have come from a one-nucleate sporocyte following a suppressed heterotypic mitosis, after which the chromosomes had split as illustrated in figure 23. The condition then followed a unipolar heterotypic spindle such as is shown in figures 20 and 21. Such a failure of the homoeotypic mitosis to distribute the halves of the 7 split chromosomes would result in a giant cell with 14 chromosomes which might become a pollen grain. A single example of such a cell was observed, figure 37, and in this case a small mass of cytoplasm had been pinched off at one side presenting behavior similar to that shown in figure 29. Through such a history might arise in haploid plants from time to time giant pollen grains produced singly in the microsporocytes and carrying two haploid sets of chromosomes. A triploid plant would result through the fertilization of a haploid egg from such a pollen grain and a tetraploid plant through the fertilization of an egg of like constitution.

DISCUSSION

The cytology of the *Datura* haploids has been described by BELLING and BLAKESLEE (1923, 1927). The 12 chromosomes show no attraction for one another at metaphase of the heterotypic mitosis in the micro-

sporocytes but either move at random to the poles (in assortments of 1 and 11, 2 and 10, 3 and 9, 4 and 8, 5 and 7, 6 and 6), or there is non-reduction. When there is segregation the groups of chromosomes after a short interphase pass through a homoeotypic mitosis where each divides and the halves are distributed in the usual manner. Such microsporocytes form 4 small microspores, usually 2 equal and smaller and 2 equal and larger. Polyspores are developed when irregularities during anaphase of the first division give chromosomes independent of the main groups and these organize very small cells in addition to the 4 principal cells. All of these small grains constitute the mass of the abortive pollen which is developed. Non-reduction takes place when the 12 chromosomes split and the halves are distributed in two sets, 12+12, and two pollen grains result each with the haploid set of chromosomes. These constitute the good pollen grains which make up about 12 percent of the total product. The good pollen grains are about the same size as the grains from the diploid *Datura*. Haploids selfed give diploids as would be expected.

The haploid *Oenothera* appears to follow the same history as the haploid *Datura*. Its 7 chromosomes show the same irregular distribution by the heterotypic mitosis in assortments of 1 and 6, 2 and 5, 3 and 4. The good pollen grains are likewise found 2 in a microsporocyte as the result of one mitosis which distributes the halves of the 7 chromosomes which split. This mitosis in *Oenothera* is the homoeotypic division, the heterotypic mitosis being suppressed because a bipolar spindle is not developed. BELLING and BLAKESLEE do not take a clear position on the homology of the single mitosis in *Datura* whether heterotypic or homoeotypic. We suspect it to be the homoeotypic mitosis as in *Oenothera* and think that they have failed to recognize the suppression of the heterotypic. This might readily happen in studies on iron-acetocarmine preparations which do not show spindle structure.

CHIPMAN and GOODSPEED (1927) give an account of meiosis in the microsporocytes of the haploid from *Nicotiana tabacum* var. *purpurea*, one of the two haploids reported by CLAUSEN and MANN (1924). This haploid (24 chromosomes) was female sterile but some viable pollen is produced. There is a stage of synizesis followed by pachynema which segments into the haploid set of chromosomes which do not pair at diakinesis. Bipolar spindles are often formed resulting in a random distribution of the 24 chromosomes some of which occasionally pass into the cytoplasm. Univalents sometimes divide during the heterotypic mitosis and when rarely all 24 divide there results a giant spindle and the formation of diads which might develop into pollen grains with the full set of

haploid chromosomes. There was some evidence of the occasional suppression of the heterotypic spindle and the gathering of the haploid set of chromosomes into a single nucleus as occurs after the unipolar spindle of our haploid *Oenothera*. A normal homoeotypic mitosis following such behavior would give diads with the full set of haploid chromosomes. A peculiar feature of the *Nicotiana* haploid, as also of the haploid wheat, is the precocious division of the univalents at heterotypic metaphase which we have not found in *Oenothera*. In other respects there seems to be a fairly close agreement in meiotic behavior between the haploid *Nicotiana* and the haploid *Oenothera*.

GAINES and AASE (1926) in their account of the haploid wheat were the first to publish a detailed description of the cytology of a haploid spermatophyte. The haploid (21 chromosomes) came from seed of a hybrid wheat No. 128, *Triticum compactum humboldtii* Kcke. (42 chromosomes) after an attempt to pollinate by *Aegilops cylindrica*. The haploid was not to be distinguished from the female parent No. 128 until the time of flowering when peculiarities characteristic of sterility appeared. The plant was about 99.8 percent seed sterile. There was no pairing of chromosomes during meiosis in the microsporocytes because there were probably three dissimilar sets of 7 chromosomes each. The chromosomes are generally distributed irregularly during the heterotypic mitosis but sometimes the chromosomes divide and the halves pass in an orderly manner to opposite poles, simulating a homoeotypic mitosis, or there may be a mixture of the two processes. The homoeotypic mitosis continues the disorderly distribution of the chromosomes giving many irregularities and forms of polyspory. No normal pollen grains were observed to develop although such might rarely be formed. An uncommon peculiarity was observed in the fusion of adjacent microsporocytes after which giant spindles may be developed including the chromosomes of two or more nuclei; these do not pair and are distributed at random. This would seem to be behavior similar to the process of "endo-duplication" described by JÖRGENSEN (1928). From such fusions come giant pollen grains which may occasionally be found in the mixture of abnormalities produced. Except for the extraordinary fusions of the microsporocytes and the early division of the chromosomes before their distribution by the heterotypic mitosis most of the irregularities of this haploid wheat are present in our haploid *Oenothera* which in addition gave us the history of the small amount of fertile pollen produced.

Very different in some important respects from the other cytological accounts of haploids is the interesting description of some *Solanum*

haploids by JÖRGENSEN (1928). The pollen tube of *Solanum luteum* may enter the embryo sac of *S. nigrum* and discharge its two sperm nuclei but these fail to fuse with the egg and endosperm nucleus and finally disintegrate. From some of the unfertilized eggs embryos begin to develop and there can scarcely be doubt that the seeds which produce haploids come from such parthenogenetic development. During meiosis in the microsporocyte the 36 chromosomes of the haploid at diakinesis show some degree of pairing, the number of pairs ranging from 3 to 11 or 12. The larger number of pairs suggests a reduction division of the 12_2+12_1 type. At the heterotypic metaphase only the bivalents are constantly present on the equatorial plate, the univalents being scattered through the cell and most often in the polar regions. The number of bivalents on the plate ranges from 3 to 12 with the numbers 5 to 8 most frequent. Univalent chromosomes lying near the plate may divide and their products can be recognized at the homoeotypic metaphase by their small size. The chromosomes pass irregularly to the poles in numbers ranging from 15 to 22 with 18 most frequent. The homoeotypic mitosis more commonly presents 18 chromosomes at metaphase; these divide and anaphase proceeds regularly giving 4 pollen grains each as a rule with 18 chromosomes. Meiosis in the megasporocyte is peculiar in that more of the chromosomes during the heterotypic mitosis pass to the micropylar end of the cell. Since the embryo sac develops from the chalazal megaspore it receives a nucleus with the smaller number of chromosomes (14 to 16) which apparently are not enough for further development since the nucellus soon breaks down. A striking peculiarity of the *Solanum* haploids is the pairing of certain chromosomes in the heterotypic mitosis. This, as JÖRGENSEN points out, suggests that the 36 chromosomes of the haploid constitute a group composed of 3 sets of 12 each of which 2 sets contain homologues of sufficient similarity to bring them together in a true synapsis. Such behavior would not be expected and does not occur in *Datura*, *Nicotiana* or *Oenothera* where the haploid count is the basic chromosome number of the genus.

The most interesting feature of the process leading to the development of the fertile pollen of the haploid *Oenothera* is the clear cut suppression of the heterotypic mitosis through failure to develop a normal spindle with the result that a reconstituted nucleus takes the seven chromosomes which then divide and the halves are distributed by a homoeotypic mitosis to form two pollen grains in each microsporocyte. There is then no reduction division and the fertile pollen carries the somatic set of seven chromosomes characteristic of the haploid. ROSENBERG (1917) first de-

scribed such a history for *Hieracium laevigatum* and *H. lacerum* and called it a "semi-heterotypic" division including under the term also the cases where chromosomes without conjugation are distributed irregularly by the heterotypic mitosis to give diads with various chromosome counts. This latter behavior is also found in the haploid *Oenothera* and gives the large mass of shriveled pollen grains present.

The term "semi-heterotypic" does not seem to us fortunate since the heterotypic mitosis either fails entirely to operate or performs irregularly; in neither case is it a half expressed division. We prefer to call the behavior of the first type suppression of the heterotypic mitosis and it is of course a very important modification of sporogenesis since it results in fertile pollen with the set of chromosomes characteristic of the parent. ROSENBERG was quick to point out that suppression of the heterotypic mitosis permits the doubling of chromosome numbers through the union of gametes carrying the somatic sets of the parents. Thus, as from fertile haploids come diploids so from diploids might come tetraploids. His earlier work was on parthenogenetic *Hieracia* but in later papers (ROSENBERG 1926, 1927) he develops this idea and suggests the possible origin of tetraploid hybrids through the union of gametes produced with non-reduction.

Material support for ROSENBERG's views on the origin of tetraploid hybrids has appeared in the interesting cross of *Raphanus* × *Brassica* recently described by KARPECHENKO (1927). The F_1 hybrids form diploid pollen grains by the suppression of the heterotypic mitosis. Tetraploid pollen grains may arise through two steps:—(1) A tetra₁ group is established by the association of two nuclei in a microsporocyt and the gathering of the two diploid sets of chromosomes through the fusion of the two heterotypic spindles; (2) The failure of the two combined heterotypic spindles to bring about segregation leads to a reconstituted nucleus with the tetraploid group, and tetraploid diads are formed following a homoeotypic mitosis. Unions of haploid, diploid and tetraploid gametes in various combinations, complicated by some numerical irregularities of chromosome distribution, give possibilities of a large assortment of polyploids which were in part realized. Among these the tetraploids from the union of diploid gametes were noteworthy as fertile plants showing no segregation and with characters of a species distinct from both parents. They show one way through which stable and fertile tetraploid hybrids may arise in sharp contrast to the "indirect chromosome binding" of WINGE (1917, 1925), and the somatic tetraploidy by "endo-duplication" of JØRGENSEN (1928). It is interesting to note how

complicated has become the subject of the origin of polyploids, with a number of possible methods probably present in different groups of plants.

Haploids carrying only non-homologous chromosomes have a peculiar interest in genetics because there can be no question of heterozygosity to complicate, if they are fertile, a study of their progeny. When selfed their seed should give homozygous diploids unless gene mutations take place or irregularities of chromosome distribution produce chromosomal sports which may be checked by cytological studies. Both chromosomal sports and gene mutations in diploids from haploids have been noted in *Datura* (BLAKESLEE and others 1927). Two gene mutations, curled and tri-carpel, have been established. They are rare and recessive and the evidence indicates that the mutations probably occur in the egg or pollen grain of the haploid parents. In the selfed lines from the first plant of pointed tips, table 1, an interesting plant, *red elongate*, with important distinguishing characters appeared in three cultures (27.41, 27.42 and 27.43). One family (28.35) has been grown from red elongate selfed; it consisted of 185 *franciscana*, 20 red elongate, 1 pointed tip and 1 broad-leaved dwarf. The result suggests irregularities of chromosome distribution and offers an interesting subject for study. Progeny from another selfed plant of pointed tips out of the cross *franciscana* × *franciscana sulfurea* dwarf, table 3 (culture 27.45), gave two remarkable plants of a wholly new type named *light green* which has pollen almost wholly perfect. From light green selfed one family (28.37) has been grown consisting of 113 plants all true to the parent type. This behavior suggests a gene mutation and it should be studied in contrast to red elongate.

SUMMARY

1. A haploid sport, named pointed tips, is thrown by the homozygous *Oenothera franciscana*. The plants are of about half the stature of *franciscana* with all organs proportionally smaller. Pollen is produced in small amounts and frequently flowers develop none at all, the anthers becoming shriveled. The pollen contains from 60 to 90 percent of shrunken and empty grains. Some fertile ovules are developed and the plant when selfed sets a small amount of seed.

2. The haploid thus far has appeared only four times in a series of generations of *O. franciscana* in a ratio of about 1:1000. It has also been thrown from crosses of *franciscana* to certain derivatives.

3. Pointed tips selfed, tables 1 and 2, gives a progeny almost wholly of the parent type *franciscana*, but a few plants of pointed tips appear and occasionally other forms. Of the latter, red elongate and light green merit attention and further study.

HAPLOID SPORT FROM OENOTHERA

4. Pointed tips backcrossed to *franciscana*, table 3, gave only *franciscana*. but in large cultures an occasional haploid would be expected.

5. Pointed tips is also recorded from a large culture of *Oenothera Hookeri*, a homozygous form very closely related to *O. franciscana*.

6. In all cases the haploid may be interpreted as arising from parthenogenetic development of an egg. This interpretation finds support in the fact that pointed tips selfed and backcrossed to *franciscana* almost wholly *franciscana* showing that its gametes must be of the *franciscana* genotype.

7. The few fertile pollen grains of the haploid are developed as result of the suppression of the heterotypic mitosis. The prophase is as in *franciscana* except that there is no pairing of the 7 chromosomes at diakinesis. The heterotypic spindle fails to attain the bipolar condition although one pole is generally evident. The chromosomes are not distributed but remain in a group and a nucleus is reconstituted which corresponds to that in the period of interkinesis of normal meiosis. In this nucleus the chromosomes split exactly as in interkinesis. Then comes homoeotypic mitosis which distributes the halves of the split chromosome to give two nuclei with 7 chromosomes each, and two fertile pollen grains are developed in each microsporocyte.

8. The large mass of shriveled and sterile pollen grains results from the irregular distribution of the 7 chromosomes by the heterotypic mitosis. Assortments of 1 and 6, 2 and 5, 3 and 4. In this manner the microsporocyte comes to contain a larger and a smaller nucleus neither of which has the full complement of 7 chromosomes. During interkinesis the chromosome splits and the halves are distributed as usual by a homoeotypic mitosis with the result that four nuclei are formed all lacking the full set of chromosomes. Cell division usually gives four small pollen grains which become shrunken and empty of contents.

9. Very rarely a homoeotypic spindle following the suppression of the heterotypic mitosis will itself fail to reach full development. In such cases the 7 split chromosomes of the period corresponding to interkinesis enter a reconstituted nucleus and a single giant pollen grain with 14 chromosomes may be produced by the microsporocyte.

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SHRIVELLED ENDOSPERM IN SPECIES CROSSES IN WHEAT, ITS CYTOLOGICAL CAUSES AND GENETICAL EFFECTS.¹

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INTRODUCTION

In many species crosses it has been found that the second and later generations are composed chiefly of individuals identical with or very similar to the pure species. The hybrids with various combinations of the parental characters, which should constitute nearly the whole F_2 if ordinary genetic principles apply, are missing or scantily represented. In general, the greater the sterility of the F_1 , the more the F_2 plants resemble the pure species. In cases in which the species differ in chromosome number this result may be attributed to the death of gametes with intermediate chromosome numbers, or to their inability to function normally owing to the unbalanced condition of the chromosomes; it may, of course, also be due to mortality among zygotes with intermediate numbers.

An additional factor was suggested by the work of THOMPSON and CAMERON (1928) on the chromosome numbers of functioning gametes of species hybrids in wheat. They observed indications that shrivelling of the endosperm of hybrid seeds might play a large part in the non-appearance of expected hybrid types, and that the shrivelling was in turn due

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to chromosome conditions in the endosperm. The present paper gives the results of an investigation to determine the exact relations among chromosome numbers, shrivelling of the endosperm, and elimination of hybrid types, in crosses between 21- and 14-chromosome species of wheat (*vulgare* series \times emmer series).

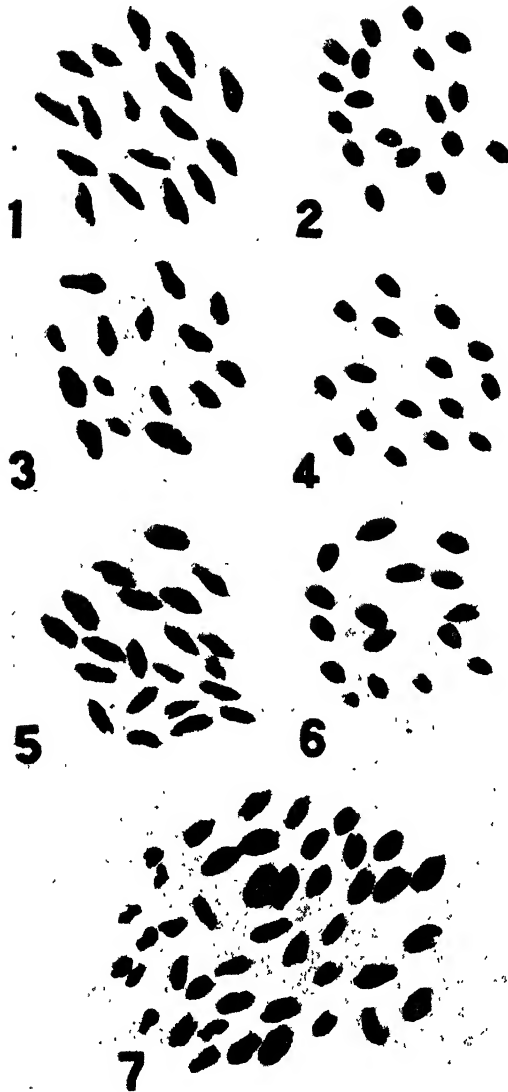
The endosperm is a unique tissue in that it results from the fusion of 2 female cells and 1 male cell. Its chromosome conditions are, therefore, different from those of any other tissue of normal plants. If the chromosome numbers of the parents are different the number present in the endosperm will depend on the direction of the cross. If the parent with the larger number is female, the endosperm will have twice the *larger* number plus the smaller; whereas if it is male, the endosperm will have twice the *smaller* number plus the larger. The totals will therefore be very different in endosperms of reciprocal crosses. Consequently, if the chromosome number has anything to do with endosperm development, we may expect to find a difference between the endosperms of reciprocal crosses.

SAX (1921) has recorded the weight of F_1 grains of several crosses between 14- and 21-chromosome wheats and their reciprocals. He found that the grains were consistently a little heavier when the 14-chromosome species was female than when it was male. He mentioned no difference between reciprocal crosses in regard to the wrinkling of grains, and, in fact, stated that in all cases they were small and wrinkled. Later, the same author (SAX 1922) reported that there is little or no correlation between the weight of F_2 seeds (borne on F_1 plants) and the height or sterility of the F_2 plants which they produced. As between the wrinkled or plump condition and F_2 sterility he reported a correlation coefficient of 0.24 when only those plants which bore heads were considered. If all sterile plants and ungerminated seeds were taken into consideration the correlation would be higher. The observations of WATKINS (1927) and of THOMPSON and CAMERON (1928) differ from those of SAX in that they found the F_1 grains to be plump if *vulgare* was used as the female parent, but invariably wrinkled if it was male. The plump seeds germinated much better than the wrinkled ones.

EXPERIMENTAL WORK

Direct Crosses, F_1

The F_1 seeds of many crosses between *vulgare* and different members of the emmer series have been examined and a consistent difference between reciprocal crosses has been observed. When the *vulgare* (21-chromosome) parent is male all the seeds are wrinkled and unhealthy in appear-



EXPLANATION OF FIGURES

FIGURE 1.—F₁ grains of *dicoccum* ♀ × *vulgare* ♂.

FIGURE 2.—F₁ grains of reciprocal cross.

FIGURE 3.—F₁ grains of *durum* ♀ × *vulgare* ♂.

FIGURE 4.—F₁ grains of reciprocal.

FIGURE 5.—Grains of (*dicoccum* × *vulgare*) F₁ ♀ × *vulgare* ♂.

FIGURE 6.—Grains of reciprocal backcross.

FIGURE 7.—F₂ Grains of *durum* × *vulgare*.

ance in every case, but when it is female they are plump and healthy, though smaller than in the pure species. The difference may be seen by comparing figure 1 (*dicoccum* ♀ × *vulgare* ♂) with figure 2 (the reciprocal) or figure 3 (*durum* ♀ × *vulgare* ♂) with figure 4 (the reciprocal). The grains illustrated are those which result directly from the cross pollination and which will grow into F₁ plants. The wrinkled grains of emmer × *vulgare* are usually slightly larger than those of the reciprocal cross. This does not indicate greater vigor but is apparently due to the fact that grain size is determined chiefly by the female parent, and pure emmer has larger grains than pure *vulgare*. Though the plump seeds of *vulgare* × emmer are slightly smaller, their greater health is shown by the fact that they germinate much better.

If many F₁ plants are required in order to carry on breeding work on a large scale there is a distinct advantage in using the *vulgare* as the female parent. This advantage more than counterbalances the fact that it is easier to get sufficient pollen from the *vulgare* parent.

The relation of chromosome number to endosperm condition in these crosses is shown in table 1. It is clear from this table that when there are 2 sets of the extra 7 *vulgare* chromosomes, the endosperm is plump (though smaller than in pure *vulgare*); but when there is only 1 set the endosperm is wrinkled. And we know from conditions in the pure species that when there are 3 sets (pure *vulgare*) or none (pure emmer) the endosperm is plump and large.

TABLE 1
Chromosome numbers and condition of endosperm in reciprocal crosses.

	<i>Vulgare</i> ♀	EMMER ♂	TOTAL	CONDITION OF ENDOSPERM
All chromosomes contributed	21+21	14	56	plump
Extra 7 <i>vulgare</i> chromosomes	7+7	0	14	
	<i>Vulgare</i> ♂	emmer ♀		
All chromosomes contributed	21	14+14	49	wrinkled
Extra 7 <i>vulgare</i> chromosomes	7	0+0	7	

When these crosses are carried on to the next generation the F₂ grains (borne on F₁ plants) present a great mixture of plump, wrinkled, shrivelled, large and small (figure 7). They have a corresponding variability in chromo-

some number. It is not possible, however, to investigate by direct microscopic examination, the exact relationship between chromosome numbers and endosperm conditions in such seeds. Nor can this be done by planting grains of the different kinds and determining the chromosome numbers of the offspring. The number of chromosomes in any plant is the sum of the number in the egg and sperm, but a knowledge of this sum does not enable us to decide how many were in the endosperm. In the case of backcrosses, however, the exact number in the endosperm can be determined indirectly. Consequently, the results obtained with later generations of direct crosses are deferred until those obtained with backcrosses are reported.

Backcrosses

Methods and Materials

In the backcross the sum of the numbers of chromosomes contributed by the two gametes can be determined (by examination of the offspring); the number contributed by one gamete (that of the pure parent) is known; consequently the number contributed by the other can be calculated. Knowing the number in each of the two gametes, it is easy to find the number in the endosperm.

Accordingly, 3 kinds of F_1 were backcrossed reciprocally with the parental types. The kinds of F_1 used were crosses of *vulgare* with *durum* (Iumillo), *dicoccum* (Vernal), and *persicum* (Black Persian). For each kind of F_1 there were four kinds of backcrosses:

- (1) F_1 female \times *vulgare* male.
- (2) F_1 " \times emmer "
- (3) *vulgare* female \times F_1 male.
- (4) emmer female \times F_1 male.

The condition of the endosperm of each grain obtained was noted (plump, wrinkled, shrivelled, large, small). Several or many of each kind were sown, and the chromosome numbers of the resulting plants were determined. This was done by means of smear preparations of pollen mother cells. It was only necessary to determine the number of unmated (univalent) chromosomes at the reduction divisions. The F_1 gamete contributes $14+x$ chromosomes where x is some number from 0 to 7. If the pure parent is emmer, its gamete contributes 14. The two sets of 14 mate in the pollen mother cells and leave the x unmated. These can be recognized by their form and behaviour. If the pure parent is *vulgare* its gamete contributes $14+7$, and the offspring has $(14+x) + (14+7)$. Again the two sets of 14 mate, the x mate with x from the 7,

leaving $7-x$ unmated. Therefore the number of univalents actually seen in this backcross will be $7-x$.

Experimental Results

Endosperm conditions in relation to the direction of crossing. The condition of the endosperm in many grains from reciprocal backcrosses is given in table 2. This classification was made by one who knew nothing of the significance of the work but was simply asked to classify the grains according to size and degree of shrivelling.

TABLE 2
Endosperm condition of seeds resulting from backcrosses.

BACKCROSS \ ENDOSPERM	LARGE PLUMP	SMALL PLUMP	LARGE WRINKLED	SMALL WRINKLED	SHRIVELLED	PERCENT OF SUCCESSFUL POLLINATIONS
<i>vulgare</i> ♀ × F_1 (<i>vulgare</i> × <i>dicoccum</i>) ♂ reciprocal	13 8	25	7 46	5 24	1 45	14.44 32.31
<i>vulgare</i> ♀ × F_1 (<i>vulgare</i> × <i>persicum</i>) ♂ reciprocal	8	13	2 71	4 20	41	9.92 35.52
<i>vulgare</i> ♀ × F_1 (<i>vulgare</i> × <i>durum</i>) ♂ reciprocal	32 19	48 7	70	10 28	7 41	33.96
<i>dicoccum</i> ♀ × F_1 ♂ reciprocal	65 28	16	27 49	33	6	30.92 36.64
<i>persicum</i> ♀ × F_1 ♂ reciprocal	21 8	7 12	11 18	5 15	3	29.50 35.33

It will be observed that in every case in which *vulgare* was the female parent the great majority of the grains are plump while in the reciprocals nearly all are wrinkled or shrivelled. Figure 5 shows a typical group of seeds of (*vulgare* × *dicoccum*) F_1 ♀ × *vulgare* ♂ and figure 6 of the reciprocal backcross. A few wrinkled seeds do occur when *vulgare* is female and a few plump ones when it is male. The results are strikingly similar for the three different kinds of F_1 (*vulgare* × *dicoccum*, *vulgare* × *persicum*, *vulgare* × *durum*). It is perhaps significant that in those cases in which nearly all the seeds are plump (*vulgare* ♀) the percentage of pollinated flowers which set seeds is low (9.92 and 14.44 percent in contrast to about 35 percent in reciprocals). This may be due to abortion and non-functioning of pollen in F_1 , though large quantities were used in pollination. It may also be possible that seeds which correspond to the wrinkled ones of other crosses cannot form at all.

The lower part of the table shows that in backcrosses with the emmer parent a much better lot of seeds is obtained when the pure parent is female. This result is in contrast to that obtained in direct crosses, for in them all seeds are wrinkled when the emmer is female.

The importance of the wrinkling and shrivelling in relation to the elimination of hybrid types becomes evident when the different kinds are planted. A large proportion of the wrinkled and shrivelled seeds fail to germinate or die at an early stage, whereas the plump ones germinate well. This is true even though the plump ones be small and the wrinkled ones large. The results are given in table 3. Of course it is possible that the same chromosome condition which makes the endosperm shrivelled may make the associated embryo weak.

TABLE 3
Germination of the different kinds of seeds.

BACKCROSS	ENDOSPERM	LARGE PLUMP	SMALL PLUMP	LARGE WRINKLED	SMALL WRINKLED	SHRIVELLED
$F_1 \text{ } \varnothing \times \textit{vulgare} \text{ } \sigma^7$	planted died			43 17	18 15	10 9
$\textit{vulgare} \text{ } \varnothing \times F_1 \text{ } \sigma^7$	planted died	20 2	27 2	7 1	12 1	
$F_1 \text{ } \varnothing \times \textit{emmer} \text{ } \sigma^7$	planted died	15 2	3 0	15 5	5 5	
$\textit{emmer} \text{ } \varnothing \times F_1 \text{ } \sigma^7$	planted died	10 1	10 0	15 2	10 2	

In connection with this table it may also be noted that in those backcrosses in which the majority of the grains are plump those which are wrinkled germinate surprisingly well. This may indicate that in these cases the wrinkling is not usually due to constitutional causes as in the other cases, but to environmental ones. Such outside influences may cause a proportion of any lot of seeds to be wrinkled.

Endosperm conditions and chromosome numbers. The results obtained in determining the chromosome numbers of the F_1 gametes which functioned to produce many backcross seeds are given in table 4. The numbers shown are those of the extra 7 *vulgare* chromosomes, so that the total number in any gamete is obtained by adding 14 to the number shown. For the backcrosses with emmers the chromosome numbers given are those actually seen as univalents; for the backcrosses with *vulgare* the

number given is 7 minus the number of the univalents seen, since, as explained under "methods," each extra chromosome contributed by the F_1 gamete will mate with one of the extra 7 from the *vulgare* gamete.

TABLE 4
Number of extra 7 *vulgare* chromosomes in F_1 reproductive cells functioning to produce various types of backcross seeds.

BACKCROSS \ ENDOSPERM	LARGE PLUMP	SMALL PLUMP	LARGE WRINKLED	SMALL WRINKLED	SHRIVELLED
<i>vulgare</i> ♀ × F_1 (<i>dicoccum</i>) ♂	6,6,7,7,	0,0,0,1,1,2,2	2,3,4,4,	2,3,3	
<i>vulgare</i> ♀ × F_1 (<i>persicum</i>) ♂	0,6,7,7,	0,0,0,1,1,2,4	1	3,3	
<i>vulgare</i> ♀ × F_1 (<i>durum</i>) ♂	5,6,7,7,7,7,7	0,0,0,0,0,0 1,1,1,2,2,3		4,4	
F_1 (<i>dicoccum</i>) ♀ × <i>vulgare</i> ♂	6,7		0,1,1,2,3	1,2,2,2, 3,3	2,2,2,4
F_1 (<i>persicum</i>) ♀ × <i>vulgare</i> ♂			1,1,2	1,3,4	3,4
F_1 (<i>durum</i>) ♀ × <i>vulgare</i> ♂	2,3,4,4,4 5,5,5,5,7		0,0,0,0,0 1,2,2,2,2,2 3,4		
<i>dicoccum</i> ♀ × F_1 ♂	0,0,0,1,1,2,2 7		5,6,6,7,7,7		
<i>persicum</i> ♀ × F_1 ♂	0,0,5	0,1	5,5,6,6		
<i>durum</i> ♀ × F_1 ♂	0,0,01		3,5,5	0,4	
F_1 ♀ × <i>dicoccum</i> ♂	0,0,0,7	2,2,3,6,7,7	2,2,3,3,3,3	3,4,4,5	
F_1 ♀ × <i>persicum</i> ♂	0,0,0,0,0	0,1,1,2		2,3,3,5	
F_1 ♀ × <i>durum</i> ♂	0,0,0,0,1,1 2,6	4,6,7		3,4	

A glance at this table is sufficient for one to observe that there is a definite relationship between the condition of the endosperm and the chromosome number of the F_1 gamete (and hence of the endosperm itself). The relationship becomes clearer when the numbers in the gametes are translated into the numbers in the endosperms. To do this it is necessary to remember that the chromosome contribution of the female gamete is doubled in the endosperm while that of the male is single. Thus in the first section of the upper row of the table, since the female *vulgare* gametes

have 7 extra chromosomes, the female contribution is 2×7 ; the male gametes have 6, 6, 7, 7. Therefore the totals are 20, 20, 21, 21. In other words there are 3 complete or nearly complete sets of 7 extra chromosomes.

TABLE 5
Usual number of extra 7 vulgare chromosomes in different types of endosperm.

ENDOSPERM BACKCROSS	LARGE PLUMP	SMALL PLUMP	LARGE WRINKLED	SMALL WRINKLED	SHRIVELLED
<i>vulgare</i> ♀ × F_1 ♂	3 sets of 7 complete or nearly so	2 complete sets	2 complete plus several	2 complete plus several	
F_1 ♀ × <i>vulgare</i> ♂	3 sets or 1 complete and 2 nearly so		1 set or 1 set and few doubled	1 set and several doubled	1 set and several doubled
emmer ♀ × F_1 ♂	0 or few single		1 set complete or nearly so		
F_1 ♀ × emmer ♂	0 or few	2 sets or few double	several doubled	several doubled	

When, in this way, the numbers throughout the table are turned into the numbers in the endosperms and the numbers for similar backcrosses combined, the total results are as shown in table 5. From this simplified table the following correlations become evident.

(1) The seeds are plump and large when the endosperm contains either (a) 3 complete or nearly complete sets of the 7 *vulgare* chromosomes or (b) none or few of them. Condition (a) is the same as or approaches that in pure *vulgare*, (b) that in pure emmer.

(2) They are plump but likely to be small when the endosperm contains 2 sets, complete or nearly so, or a double dose of one or 2 chromosomes.

(3) They are wrinkled when there is 1 set, complete or nearly so.

(4) The shrivelling is increased by the doubling of incomplete sets.

(5) If the 7 be designated a, b, c, etc., the addition of, say, a and b, does not usually produce much effect, but 2a's and 2b's usually have a pronounced effect. Also 3a's and 3b's have a great effect even when one set is complete.

Of course there are individual cases which do not fit into the scheme but this is to be expected since environmental factors also induce wrinkling,

and there may be differences depending on which of the 7 are present or absent.

Another important point which affects the proportion of plump and shrivelled seeds is the failure of many gametes to function. THOMPSON and CAMERON (1928) and SAX (1928) have shown that most of the F_1 male gametes which produce offspring have 0 or 1 *vulgare* chromosomes; some have 6 or 7; very few have intermediate numbers. The female F_1 gametes are less subject to elimination. Table 4 confirms these findings. It must be noted, however, that the plants studied are not a representative sample; on the contrary disproportionately large numbers of wrinkled and shrivelled seeds were deliberately chosen.

On the basis of proportions of functioning gametes with different chromosome numbers, as reported by these authors and confirmed in this paper, the relative frequencies of the main kinds of chromosome combinations in the endosperm and therefore of the degrees of shrivelling, is shown in table 6 for direct and backcrosses. In this table, x is between 1 and 6.

TABLE 6

CROSS OR BACKCROSS	ENDOSPERM				
	THE 7 <i>vulgare</i> CHROMOSOMES			FREQUENCY	CONDITION
	FEMALE	MALE	TOTAL		
<i>vulgare</i> ♀ × <i>emmer</i> ♂	7+7	0	diploid	all	plump
<i>emmer</i> ♀ × <i>vulgare</i> ♂	0+0	7	haploid	all	wrinkled
<i>vulgare</i> ♀ × F_1 ♂	7+7	0	diploid	many	plump
	7+7	7	triploid		plump
	7+7	x	diploid + x	few	wrinkled
F_1 ♀ × <i>vulgare</i> ♂	7+7	7	triploid	rare	plump
	0+0	7	haploid	some	wrinkled
	$x+x$	7	haploid + 2 x	some	wrinkled or shrivelled
<i>emmer</i> ♀ × F_1 ♂	0+0	0	absent	many	plump
	0+0	7	haploid	some	wrinkled
	0+0	x	x	few	wrinkled
F_1 ♀ × <i>emmer</i> ♂	7+7	0	diploid	rare	plump
	0+0	0	absent	some	plump
	$x+x$	0	2 x	many	wrinkled or shrivelled

Direct Crosses, F₂

It has already been pointed out that the exact chromosome number in the endosperm of an F₂ seed cannot be determined by finding the number in the plant which it produces. The latter is the sum of the number in the two gametes, whereas one needs to know the number in each gamete. Nevertheless, some information can be obtained in that way and it was considered desirable to secure whatever information was obtainable, even though it was inexact. Accordingly the chromosome situation was determined in a number of F₂ plants of *vulgare* × *dicoccoides*, which grew from plump and from wrinkled seeds. The results are given in table 7.

In so far as these results can be interpreted, they agree with those already described in this paper, and with the results previously reported regarding the chromosome numbers of functioning germ cells. In those cases in which the plant had 21 chromosomes (bivalents + univalents) and the seed was plump, it was probably the egg which contributed the 7 *vulgare* chromosomes; but where the seed was shrivelled it was probably the sperm which contributed the 7. Where the seed was wrinkled though the extra chromosomes were few the latter were probably contributed by the egg.

TABLE 7
Chromosome numbers of F₂ vulgare × *dicoccoides* produced by different kinds of seeds.

SEEDS CHROMOSOMES		PLUMP	LARGE WRINKLED	SMALL WRINKLED
Bivalents	Univalents			
14	0	5		
14	1	4		1
14	2	4	1	1
14	3	2	2	3
14	4		1	3
14	5		1	2
14	6	2		1
14	7 =	1		
15	6		1	
16	5	2		1

DISCUSSION

From the results reported it is clear that the condition of the endosperm depends on its chromosome content, and that many zygotes must fail to develop because of the chromosome combinations in their endosperms. In general the endosperm is plump when the 7 *vulgare* chromosomes

are absent or diploid or triploid or nearly so. It is likely to be wrinkled or shrivelled when (a) haploid for all or many of the 7, (b) diploid or triploid for some only. The farther the chromosome situation departs from the complete absence or diploidy or triploidy of the *vulgare* chromosomes the more severe is the shrivelling.

The results throw light on the non-appearance of many theoretically expected chromosome and genetic types in F_2 and later generations of ordinary crosses. The types which fail to appear are for the most part those which would be expected to have shrivelled endosperms when they were seeds, and therefore fail to germinate. Furthermore, the injurious effect on the endosperm is likely to be more severe in direct crosses than in backcrosses since in the latter the contribution of one parent must be either a complete set or none at all, whereas in ordinary hybrids both male and female gametes may contribute incomplete sets. And in the missing types both must contribute such incomplete sets.

TABLE 8

Chromosome conditions in the endosperm of seeds from which some typical missing hybrids would have to develop.

HYBRIDS WITH	<i>vulgare</i> CHROMOSOMES (a, b, c, AND SO FORTH) IN		
	FEMALE NUCLEI	MALE NUCLEUS	ENDOSPERM
16 bivalents	$2(a+b)$	$a+b$	$3a+3b$
16 bivalents+1 univalent	$2(a+b)$	$a+b+c$	$3a+3b+c$
17 bivalents+2 univalents	$2(a+b+c)$	$a+b+c+d+e$	$3a+3b+3c+d+e$

KIHARA (1924) showed that all the hybrids which develop (other than those with 14 bivalents only) have the chromosome formula $(14+x)$ bivalents + $(7-x)$ univalents. If there are more than 14 bivalents there are enough univalents to make the total 21. A complete set of the 7 *vulgare* ones must be present. A missing type with $14+x$ bivalents would have to result from the fusion of 2 gametes both of which had the same *vulgare* chromosomes (x in number). The endosperm would therefore have three of each kind represented in the x . If there were univalents in addition to the $14+x$ bivalents the endosperm condition would still be very much unbalanced unless there were enough univalents to complete the set of 7. A few examples of the missing types and the chromosome conditions in their endosperms are shown in table 8, a, b, c, etc. representing *vulgare* chromosomes. Such endosperms are likely to be more severely shrivelled (if they can develop at all) than any which can be produced in

backcrosses since in the latter none can have three of one kind unless it has a complete set.

It is possible, of course, that the unbalanced chromosome condition which injuriously affects the endosperm also affects the embryo independently of the endosperm. It may be expected, however, that the triploid endosperm would be more severely affected than the diploid embryo. If there is this independent effect, such embryos are subject to a double menace, (1) the unbalance in their own chromosome complement and (2) the shrivelled endosperm.

In practical breeding of wheat, carried on in order to produce better varieties, special attention should be paid to the shrivelled seeds. The natural tendency is consciously or unconsciously to select the better seeds for planting. But this obviously does not give a random sample of the different genotypes. In any study in which an attempt is made to analyse the genetic situation in order to guide practical efforts, special attention must be given to the poor seeds. From the practical standpoint the shrivelled seeds are just those in which the combinations of characters desired by the breeder are likely to occur. The plump ones are likely to have the complete *vulgare* set of chromosomes or none of them. Moreover, if they are planted under ordinary field conditions most of the shrivelled ones will fail to germinate. Planting in the greenhouse under the best controlled conditions of temperature, soil, moisture, etc. followed by transplanting to the field, would appear to be desirable.

The attention of practical breeders may also be directed to the superiority of the backcross. By its use types can be obtained which would never appear in direct crosses. Without the use of the backcross a real genetic analysis of species crosses in wheat would appear to be out of the question. Genetic conclusions based on the small proportion of chromosome combinations which appear in direct crosses would seem to be quite unreliable. In the backcross a larger proportion of the gametes, particularly the female ones, function.

The conclusions drawn from this work on crosses between 14- and 21-chromosome wheats may be illuminating also with respect to other crosses. The present writer has found it extremely difficult to cross *T. monococcum* (7 chromosomes) with species of the emmer series when the former is female but quite easy when it is male. Numerous attempts to cross *monococcum* female with members of the *vulgare* series yielded no results, but the reciprocal cross was occasionally successful. BLEIER's recent reviews of the literature (1928) show that other investigators have had similar experiences. When the parent with the larger chromo-

some number is female all its chromosomes are represented twice in the endosperm, whereas if it is male they are single. In crosses between a 7-chromosome species of *Aegilops* (*A. speltioides*) and various species of wheat exactly similar results have been obtained. Also it is well known that rye which has 7 chromosomes readily serves as male parent but not as female in crosses with certain varieties of *vulgare* wheat. MEISTER and TJUMJAKOFF (1928) are the only investigators who have had any success in using rye as female parent in this cross and they report only 2.5 percent successful pollinations, whereas in the reciprocal they found more than 60 percent successful.

Outside the cereals reports of species crosses which give sufficient information are not numerous enough to enable one to decide whether these conclusions are of general application. Reports have been noted, as in connection with *Nicotiana* (EAST 1928), of cases in which the species with the larger chromosome number must serve as female if success is to follow. In other cases there appear to be exceptions, but it is possible that in these cases the seeds are small and the endosperm does not play as important a part as in cereals.

SUMMARY

1. In crosses between 14- and 21-chromosome wheats the F_1 grains are plump when the 21-chromosome species is female and wrinkled when it is male. In the former case the endosperm of the seed is diploid with respect to the extra 7 *vulgare* chromosomes, and in the latter case haploid.

2. In backcrosses of F_1 with *vulgare* the great majority of seeds are plump when *vulgare* is female, though wrinkled ones occur; in the reciprocal nearly all are wrinkled or badly shrivelled. In backcrosses with emmers also the seeds are better when the pure parent is female.

3. The chromosome numbers of many backcross plants produced by different kinds of seeds (plump, wrinkled, shrivelled, large, small) were determined. This was done for three kinds of F_1 backcrossed with their *vulgare* parent, male and female, and with their emmer parent, male and female. From the number found the number in the endosperm of the seed from which each plant grew was calculated. The number in the endosperm was then compared with the physical condition of the endosperm.

4. The endosperm is plump and large when it contains (a) none or few of the extra 7 *vulgare* chromosomes, (b) 3 sets of 7, complete or nearly so. It is usually plump and small when it contains 2 sets, complete or nearly so. It is wrinkled or shrivelled when (a) it is haploid for all or many of the 7, (b) diploid or triploid for some only. The farther the chromosome

situation departs from the complete absence or complete diploidy or triploidy of the *vulgare* chromosomes, the severer is the shrivelling.

5. Endosperm conditions, depending in this way on chromosome conditions, play a large part in the non-appearance of many types in F_2 and later generations of ordinary crosses. The missing plants would have such chromosome conditions that the endosperm of the seeds from which they would have to develop would be badly shrivelled (or absent).

6. In genetic analysis and economic work on such crosses special care in selection and culture should be given to shrivelled seeds since they represent cytological and genetical types not found among the plump ones.

7. The advantages of using the parent with the larger chromosome number as the female, and of backcrosses are emphasized.

8. Apart from these crosses between *vulgare* and emmer wheats, differences in the success or other results of reciprocal crosses in general may be due in part to endosperm conditions.

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A LETHAL FACTOR IN CREPIS EFFECTIVE ONLY IN AN INTERSPECIFIC HYBRID*

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HISTORICAL INTRODUCTION

BABCOCK and COLLINS (1920, a and b) described hybrids between *Crepis capillaris* (L.) Wallr. ($n=3$) and *C. tectorum* L. ($n=4$) which died after developing to the cotyledon stage. Similar results were obtained by NAWASCHIN (1926) but later (1927) he reported a spontaneous *capillaris-tectorum* hybrid which developed normally. He suggested that the phenomenon might be associated with heterozygosity of the parents and later he obtained viable artificial hybrids (unpublished data). *C. capillaris* and *C. tectorum* are taxonomically more closely related than some other species of the genus which produce vigorous interspecific hybrids and it was an interesting result when early attempts to cross them gave inviable seedlings only.

Following the investigations of BABCOCK and COLLINS, Professor BABCOCK's assistant, Mr. C. W. HANEY, in 1924 again crossed these two species, and out of the five populations which he obtained, four contained 69 hybrids all of which died, but a fifth was made up of 40 hybrids, 20 of which died at the cotyledon stage but 20 matured as large vigorous hybrids. Figure 1 shows the parental species and one of these F_1 hybrids at maturity. The hybrids were intermediate or more like *capillaris* in habit of growth but in most other characters were more like *tectorum*. They were nearly sterile.

In the summer of 1926 the writer was given the material to determine if possible whether there was a factorial basis for the phenomenon just de-

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scribed. The investigation was carried out in the laboratories of the Division of Genetics, UNIVERSITY OF CALIFORNIA. The writer wishes to express her thanks to Mr. C. W. HANEY for his advice and assistance in the culture of plants as well as for the data given above. The plan of investigation was suggested by Professor E. B. BABCOCK and it is a pleasure for the



FIGURE 1.—Typical plants of *C. tectorum*, F_1 *C. capillaris-tectorum* and *C. capillaris* at maturity.

writer to acknowledge her indebtedness to him and to Professor J. L. COLLINS for the material and for helpful advice throughout the course of the work.

MATERIALS AND METHODS

Crepis is a genus of the Cichorieae tribe of the Compositae. *C. capillaris*, outstanding among flowering plants for its small chromosome number, belongs to the same section (Eucrepis) of the genus as *C. tectorum* (BABCOCK and LESLEY 1926). They differ chiefly in leaf shape, habit of growth, flower color, and shape and size of the achenes.

The *capillaris* plant (X.23.1) used in the cross which gave half viable hybrids was a stray plant picked up in the greenhouse by Mr. HANEY and is probably from one of the European strains. The designation X has been kept for the progeny of this plant throughout the investigation. It gave

rise to a particularly vigorous and early-flowering strain, producing under favorable conditions many branches bearing a large number of heads (figure 1). Most plants fruit well in the greenhouse or outdoors. The *teclorum* plant (T.23.1498-4) was one grown from original seed obtained from the COPENHAGEN BOTANICAL GARDEN, and its progeny have been designated by the accession number 1498. It is a vigorous uniform strain and sets fruit fairly well under bags.

The method of pollination used was a modification of that described by COLLINS (1922). The ligules and stamen tubes were removed from the outer row of florets on a head shortly after these florets opened. The unopened florets were removed and the pollen was washed from the styles with a stream of water from a dentist's chip blower. The head was then encased in a light paper bag to dry and to await pollination when the stigmatic branches should be separated. Pollination was usually carried out on the same or the succeeding day by rubbing the stigmas with opened heads of the plant to be used as a male parent. In the first season of crossing (1927) unprotected heads which had opened only the morning they were used were occasionally selected to provide pollen, but experience showed that even in that short time contamination might have taken place and in later investigations only heads protected by bags were used. The pollinated heads were again bagged and removed when the achenes were mature, about three weeks later.

The number of selfed plants obtained by this method of hybridization with *capillaris* as the female parent was small, averaging a little more than 2 percent in 1927 when 34 pure *capillaris* and 1664 F₁ hybrids were obtained. Seventeen of these *capillaris* plants comprised two whole populations. This does not include the four haploid plants of *C. capillaris* which were found in these cultures. A preliminary note on the occurrence of two of them has been published (HOLLINGSHEAD 1928), and it is hoped to report on them in detail in a later paper. The cross was usually made on *capillaris* since it was early learned that the reciprocal (at least the one involving the 1498 strain of *teclorum*) was only rarely successful and that selfed progeny occurred relatively more often. Only six of the *capillaris-teclorum* hybrids recorded in this paper were from achenes produced on *teclorum* plants. BABCOCK and COLLINS (1920 a and b) showed that the reciprocal hybrids obtained in their investigations were inviable and those obtained by the writer have been grouped together in the data given below.

The progeny of the original *teclorum* plant, T.23.1498-4, were from achenes produced on the plant while isolated from other *teclorum* plants in the greenhouse. All other *teclorum* 1498 seed was taken from bagged, or in

one instance, caged plants. The achenes were planted in sterilized sand and the very young seedlings were transplanted to flats or pots.

INVESTIGATIONS INVOLVING THE 1498 STRAIN OF TECTORUM

When the writer took up the work the problem was to determine (1) whether the difference between the normal and abnormal hybrids was due to one or more factors, and (2) if so, from which parent they came. The *capillaris* parent of the hybrid population of which half grew to maturity was also the parent of another population of 57 hybrids all of which died. This indicated that it was perhaps the *tectorum* parent which was responsible for the different behavior of this population. The 1:1 ratio of viable to inviable hybrids suggested that this plant might have been heterozygous for a lethal factor which killed the interspecific hybrid into which it entered. To test this hypothesis it was planned to grow a progeny of this *tectorum* plant and to cross each individual of it to *capillaris*. If the *tectorum* parent had been heterozygous for an interspecific lethal the progeny should be segregating for it and the genotypic constitution of each plant should be indicated by the kind of hybrids it produced when crossed to *capillaris*.

When the investigation was begun in August 1926 there were growing a number of *tectorum* plants, T.26.1498-1 to 30, progeny of this original *tectorum* T.23.1498-4. Most of them were dead or dying but a few, after being cut back, produced some more heads. They were used chiefly as male parents in crosses with *capillaris*, and some hybrid achenes were obtained. Three strains of *capillaris* were used but most of the crossing involved a plant of the X strain. The possibility that the *capillaris* parent might have something to do with the inviability of the hybrid offspring was constantly in mind throughout the early part of the work and this was the chief reason why the X strain, the one involved in the cross which first gave viable hybrids, was the one chiefly used. Evidence to be given later indicated that the *capillaris* plants used had no effect on the viability of the hybrid offspring and in the tables the hybrids are grouped without regard to the *capillaris* parent.

The achenes obtained from these first crosses were planted in December, 1926, but unfortunately the young plants were attacked by red spiders and in a few cases it was difficult to tell whether the seedlings died because of the spiders or whether they were exhibiting the phenomenon which had come to be known as "dying at the cotyledon stage." The results of this preliminary work are given in the first four lines of table 1. It was suspected that those hybrids in the progenies of T.26.1498-26 and T.26.1498-

24 which died owed their death to the spider attack and this was substantiated by investigations to be described later.

Hoping to obtain plants for crossing during the winter months another lot of achenes from the original *tectorum* T.23.1498-4 was planted in August, 1926 and grown in the greenhouse under the numbers T.26.1498-60 to -95 and at the same time some *capillaris* strains were planted. The *tectorum* plants which first sent up flower stalks were attacked by spiders and did not prove to be very vigorous, while the *capillaris* had not yet bloomed. It was decided that conditions were unfavorable for hybridization during the winter and the plants were put outside in the hope of keeping them till spring. Most of them died but a few bloomed late enough in the spring to be available for crossing. The last of the achenes from the original *tectorum* plant were sown in February, 1927, and the plants were grown under the numbers T.27.1498-21 to 40. The population was a vigorous rather uniform one with no outward sign of a lethal factor present in any of the plants.

This provided in June, 1927, a group of *tectorum* plants, progeny of the original T.23.1498-4, each of which was crossed to *capillaris*. An effort was made to obtain at least twenty hybrid achenes from each *tectorum* parent to ensure a progeny large enough to make reasonably valid the indicated parental genetic constitution. As table 1 shows, many more than twenty were obtained in most cases, but low germination in some instances greatly reduced the number of hybrids obtained below the number expected. Variation in percentage of germination characterized the whole investigation and in a few cases the number of hybrids obtained was too small to indicate the parental constitution and these have been omitted in this table.

The results obtained were in agreement with the hypothesis that this population of *tectorum* plants was segregating for a lethal factor effective in an interspecific hybrid but without effect on *tectorum*. The results are summarized in table 1. The hybrids are grouped with respect to the *tectorum* parent and each group will be described as a "population" although in most cases the hybrids in a group were obtained from two or more *capillaris* plants. The footnotes to this table and to those which follow explain why a few hybrids were not classified. The one case here attributed to experimental error is probably due to contamination of heads used for pollination. As the table shows, some *tectorum* plants gave hybrids, all of which lived, indicating no lethal in the parent (*L L*). A larger group gave hybrids, part of which lived and part of which died, indicating a parent heterozygous for the lethal (*L l*). Some gave hybrids, all of which died,

indicating parents homozygous for the lethal (*ll*). The letters *l*, for the lethal, and *L*, for the allelomorph, have been assigned arbitrarily and do not indicate any dominant or recessive relationships.

TABLE 1

F₁ hybrids obtained by crossing progeny of original C. tectorum plant T.23.1498-4 to capillaris.

tectorum PARENT	F ₁ ACHENES	GERMINATED	UNCLAS- SIFIED ³	LIVED	DIED	GENOTYPE (C tectorum PARI
T. 26. 1498-26	26	21		18	3	<i>L L</i> ¹
" " -24	32	13		12	1	<i>L L</i> ¹
" " -19	14	9		0	9	<i>l l</i>
" " -23	18	7		0	7	<i>l l</i>
T. 26. 1498-63	98	58		0	58	<i>l l</i>
" " -68	39	25		0	25	<i>l l</i>
" " -77	37	15		15	0	<i>L L</i>
" " -78	24	15		15	0	<i>L L</i>
" " -82	43	15		15	0	<i>L L</i>
" " -84	40	18	1	17	0	<i>L L</i>
" " -86	9	7		5	2	<i>L l</i>
" " -88	48	40		0	40	<i>l l</i>
" " -91	26	12	1	11	0	<i>L L</i>
T. 27. 1498-21	44	39	1	16	22	<i>L l</i>
" " -22	45	32		32	0	<i>L L</i>
" " -23	47	22		18	4	<i>L l</i>
" " -24	29	11		11	0	<i>L L</i>
" " -26	27	13		0	13	<i>l l</i>
" " -27	54	45		20	25	<i>L l</i>
" " -28	26	18		9	9	<i>L l</i>
" " -29	23	23		10	13	<i>L l</i>
" " -30	32	16	1	7	8	<i>L l</i>
" " -31	71	47	1	0	46	<i>l l</i>
" " -32	28	21		7	14	<i>L l</i>
" " -34	36	30		12	18	<i>L l</i>
" " -35	34	22		12	10	<i>L l</i>
" " -36	43	23		0	23	<i>l l</i>
" " -37	51	30		15	15	<i>L l</i>
" " -38	24	14		5	9	<i>L l</i>
" " -39	23	8		4	4	<i>L l</i>
" " -40	32	12		11	1 ²	<i>L L</i>

LL : Ll : ll

10: 13: 8

¹ Red spiders attacked the young plants. Progeny tests (see tables 2 and 3) showed the *tectorum* parents were free from the lethal.

² Probably due to experimental error, the chances of obtaining such a ratio from a heterozygous parent being 3 in 1000.

³ Did not survive transplanting.

As noted above two of the *tectorum* plants tested in 1926 gave hybrids most of which lived, and presumably those which died owed their death to red spiders. In order to prove that these two plants were really free from the lethal, progenies of each of them were grown in 1927 and crossed to *capillaris*. The results are summarized in tables 2 and 3. Nine plants of the progeny of T.26.1498-26 gave 148 hybrids, all living, indicating they were free from the lethal. The chance of obtaining nine homozygous plants from a heterozygous parent is only 1 in 512 so that it is most probable that the *tectorum* grandparent of these hybrids, T.26.1498-26, was free from the lethal and it has been so classified. Fifteen plants of the progeny of T.26.1498-24 gave 292 hybrids of which only one died. It was in a population of 25 hybrids and has been attributed to experimental error. A progeny test of its parent, T.27.1498-11 (table 4), showed no evidence of a lethal. These results indicate that the *tectorum* grandparent, T.26.1498-24, was also free from the lethal.

TABLE 2
F₁ hybrids obtained by crossing progeny of T.26.1498-26 to capillaris.

<i>tectorum</i> PARENT	F ₁ ACHENES	GERMINATED	UNCLAR- SIFIED	LIVED	DIED	GENOTYPE OF <i>tectorum</i> PARENT
T. 27 1498-41	50	23		23		<i>L L</i>
" " -42	25	17		17		<i>L L</i>
" " -43	21	17		17		<i>L L</i>
" " -45	47	27		27		<i>L L</i>
" " -46	27	7		7		<i>L L</i>
" " -47	14	6		6		<i>L L</i>
" " -48	33	28		28		<i>L L</i>
" " -49	39	15		15		<i>L L</i>
" " -50	20	8		8		<i>L L</i>
Total	276	148		148		

The genotypes of the progeny of the original *tectorum* plant having been established the distribution proved to be 10 *LL* : 13 *Ll* : 8 *ll*. The expected distribution for a single factor difference would be 7.75 *LL* : 15.5 *Ll* : 7.75 *ll*. Such a difference between the actual and the expected as tested by the χ^2 method would occur six times in ten by random sampling and the distribution can therefore be accepted as agreeing with the theoretical expectation.

In order to further establish the conclusion that we were dealing with a simple lethal factor which did not affect the *tectorum* plant bearing it but which caused the death of the interspecific hybrid into which it entered

another season's work was planned. The genotypes of the various *lectorum* plants having been determined and achenes having been obtained from

TABLE 3
F₁ hybrids obtained by crossing progeny of T.26.1498-24 to capillaris.

<i>lectorum</i> PARENT	F ₁ ACHENES	GERMINATED	UNCLAS- SIFIED ¹	LIVED	DIED	GENOTYPE OF <i>lectorum</i> PARENT
T. 27.1498- 2	23	9		9		<i>L L</i>
" " - 3	27	16		16		<i>L L</i>
" " - 4	42	14	1 ¹	13		<i>L L</i>
" " - 5	47	27		27		<i>L L</i>
" " - 6	23	22		22		<i>L L</i>
" " - 8	25	10		10		<i>L L</i>
" " - 9	22	12		12		<i>L L</i>
" " -10	35	25		25		<i>L L</i>
" " -11	37	25		24	1 ²	<i>L L</i>
" " -12	59	28	1 ³	27		<i>L L</i>
" " -14	20	18		18		<i>L L</i>
" " -15	37	17		17		<i>L L</i>
" " -16	37	26		26		<i>L L</i>
" " -18	32	18		18		<i>L L</i>
" " -20	45	27		27		<i>L L</i>
Total	511	294	2	291	1	

¹ Abnormal growing point and thick leathery leaves. Died very early.

² Doubtless due to experimental error.

³ Did not survive transplanting.

most of them, two of each genotype were chosen to provide progeny for further work. The six plants and their progenies were as follows:

<i>Genotype</i>	<i>Plant</i>	<i>Progeny</i>
<i>L L</i>	{ T. 27.1498-11	28H.T.1498D-1 to-24
	{ T. 27.1498-15	28H.T.1498E-1 to-16
<i>L l</i>	{ T. 27.1498-30	28H.T.1498F-1 to-24
	{ T. 27.1498-34	28H.T.1498G-1 to-32
<i>l l</i>	{ T. 27.1498-31	28H.T.1498H-1 to-24
	{ T. 27.1498-36	28H.T.1498 I-1 to-16

As many plants as possible of each progeny were crossed to *capillaris* in the summer of 1928. The achenes obtained were sown in the fall of 1928 and the results obtained are summarized in tables 4 to 9.

The progeny of the first *L L* plant, T.27.1498-11, gave 22 hybrid populations containing 249 plants all developing normally (table 4). Seventeen

of these populations contained at least six hybrids, the smallest number from which it is possible to determine the parental genotype with any degree of certainty (the chance of obtaining six viable hybrids from a heterozygous parent is 1 in 64), and these were classified *L L*, while the nature of the other five populations with fewer hybrids was in agreement with the expectation that all the *tectorum* plants of this group were *L L*. The

TABLE 4

F₁ hybrids obtained by crossing progeny of T.27.1498-11 (L L) to capillaris.

<i>tectorum</i> PARENT	F ₁ ACHENES	GERMINATED	UNCLAS- SIFIED	LIVED	DIED	GENOTYPE OF <i>tectorum</i> PARENT
28H.T.1498D- 1	15	6		6		<i>L L</i>
" " - 2	27	22		22		<i>L L</i>
" " - 3	2	2		2		
" " - 4	15	13		13		<i>L L</i>
" " - 5	14	12		12		<i>L L</i>
" " - 6	27	10		10		<i>L L</i>
" " - 8	18	16		16		<i>L L</i>
" " - 9	10	10		10		<i>L L</i>
" " -10	10	7		7		<i>L L</i>
" " -12	11	9		9		<i>L L</i>
" " -13	29	13		13		<i>L L</i>
" " -14	16	5		5		
" " -15	25	24		24		<i>L l</i>
" " -16	26	17		17		<i>L L</i>
" " -17	19	11		11		<i>L L</i>
" " -18	9	8		8		<i>L L</i>
" " -19	8	5		5		
" " -20	20	14		14		<i>L L</i>
" " -21	23	21		21		<i>L L</i>
" " -22	9	5		5		
" " -23	21	15		15		<i>L L</i>
" " -24	5	4		4		
Total	359	249		249		

progeny of the second *L L* plant, T.27.1498-15, gave 10 hybrid populations including in all 149 hybrids, all normal (table 5). Eight of these populations contained enough hybrids to enable the parents to be classified as normal. This confirms the expectation that plants genetically *L L* give only *L L* progeny and that races were isolated free from the lethal.

The progeny of T. 27.1498-30 (*L l*) were segregating for the lethal as shown by the occurrence again of hybrid populations of three types: all living; part living, part dying; all dying (table 6). The distribution was 6 *L L* : 11 *L l* : 7 *ll* a close approximation to the expected 6 *L L* : 12 *L l*

TABLE 5
F₁ hybrids obtained by crossing progeny of T.27.1498-15 (L L) to capillaris.

<i>tectorum</i> PARENT	F ₁ ACHENES	GERMINATED	UNCLAS- SIFIED	LIVED	DIED	GENOTYPE OF <i>tectorum</i> PARENT
28H.T.1498E- 2	32	26		26		L L
" " - 4	19	18		18		L L
" " - 5	2	2		2		
" " - 8	53	46		46		L L
" " -10	10	5		5		
" " -11	15	12		12		L L
" " -12	11	8		8		L L
" " -14	28	17		17		L L
" " -15	24	9		9		L L
" " -16	6	6		6		L L
Total	200	149		149		

TABLE 6
F₁ hybrids obtained by crossing progeny of T.27.1498-30 (L l) to capillaris.

<i>tectorum</i> PARENT	F ₁ ACHENES	GERMINATED	UNCLAS- SIFIED ¹	LIVED	DIED	GENOTYPE OF <i>tectorum</i> PARENT
28H.T.1498F- 1	19	6		6	0	L L
" " - 2	24	11		4	7	L l
" " - 3	23	16		10	6	L l
" " - 4	27	12		9	3	L l
" " - 5	22	14		6	8	L l
" " - 6	21	15		0	15	l l
" " - 7	28	13		0	13	l l
" " - 8	17	14		14	0	L L
" " - 9	14	9		6	3	L l
" " -10	26	13		0	13	l l
" " -11	19	14		4	10	L l
" " -12	36	24		11	13	L l
" " -13	33	15		15	0	L L
" " -14	17	6		6	0	L L
" " -15	27	24		13	11	L l
" " -16	31	10		0	10	l l
" " -17		7		2	5	L l
" " -18		14		0	14	l l
" " -19		9	1	0	8	l l
" " -20	28	13		9	4	L l
" " -21	22	18		18	0	L L
" " -22	22	11		11	0	L L
" " -23	24	7		5	2	L l
" " -24	21	11		0	11	l l

L L: L l: l l

6: 11: 7

¹ Did not survive transplanting.

: 6 *ll*. Similarly the progeny of T.27.1498-34 (*L l*) showed a distribution 4 *LL* : 19 *L l* : 4 *ll* (table 7). Such a deviation from the expected 6.75 *LL* : 13.5 *L l* : 6.75 *ll* would occur 11 times in 100 by random sampling and the distribution is therefore in agreement with expectation.

TABLE 7
F₁ hybrids obtained by crossing progeny of T.27.1498-34 (L l) to capillaris.

<i>lectorum</i> PARENT	<i>F₁</i> ACHENES	GERMINATED	UNCLAS- SIFIED	LIVED	DIED	GENOTYPE OF <i>lectorum</i> PARENT
28H.T 1498G- 1	20	12		7	5	<i>L l</i>
" " - 2	26	20		0	20	<i>l l</i>
" " - 3	23	5		2	3	<i>L l</i>
" " - 5	10	8		3	5	<i>L l</i>
" " - 7	29	17		17	0	<i>LL</i>
" " - 9	25	7		3	4	<i>L l</i>
" " -10	23	5		2	3	<i>L l</i>
" " -11	18	14		9	5	<i>L l</i>
" " -12	15	13		0	13	<i>l l</i>
" " -13	31	17		9	8	<i>L l</i>
" " -14	19	4		3	1	<i>L l</i>
" " -15	43	18		9	9	<i>L l</i>
" " -16	29	6		6	0	<i>LL</i>
" " -17	45	26		26	0	<i>LL</i>
" " -18	43	13		7	6	<i>L l</i>
" " -19	33	32	1 ¹	14	17	<i>L l</i>
" " -20	35	13		7	6	<i>L l</i>
" " -21	29	19		9	10	<i>L l</i>
" " -22	13	12		6	6	<i>L l</i>
" " -23	20	3		1	2	<i>L l</i>
" " -24	19	8		4	4	<i>L l</i>
" " -25	21	16		0	16	<i>l l</i>
" " -26	30	13		7	6	<i>L l</i>
" " -27	30	21	1 ²	20	0	<i>LL</i>
" " -28	21	16		5	11	<i>L l</i>
" " -30	20	11		9	2	<i>L l</i>
" " -32	21	7		0	7	<i>l l</i>

LL: *L l*: *ll*

¹ Did not survive transplanting.

4: 19: 4

² Abnormal, died early.

The progeny of T.27.1498-31 (*l l*) gave 19 hybrid populations containing 157 plants all of which were inviable (table 8). Eleven of these contained enough hybrids to enable the parents to be classified as *l l*. The progeny of T.27.1498-36 (*l l*) gave 14 hybrid populations containing 185 seedlings, all of which died (table 9). Twelve of these contained enough hybrids to enable the parents to be classified as *l l*. Here races were established homozygous for the lethal.

TABLE 8
F₁ hybrids obtained by crossing progeny of T.27.1498-31 (l l) to capillaris.

tectorum PARENT	F ₁ ACHENES	GERMINATED	UNCLAS- SIFIED	LIVED	DIED	GENOTYPE OF tectorum PARENT
28H.T.1498H- 2	17	12			12	l l
" " - 3	10	7			7	l l
" " - 4	17	13			13	l l
" " - 5	5	4			4	
" " - 6	9	9			9	l l
" " - 7	17	13			13	l l
" " - 8	12	11			11	l l
" " - 9	14	13			13	l l
" " -11	7	3			3	
" " -12	13	11			11	l l
" " -13	23	9			9	l l
" " -14	4	1			1	
" " -15	27	19			19	l l
" " -16	27	1			1	
" " -17	22	18			18	l l
" " -19	2	2			2	
" " -21	7	5			5	
" " -23	2	1			1	
" " -24	7	5			5	
Total	242	157			157	

TABLE 9
F₁ hybrids obtained by crossing progeny of T.27.1498-36 (l l) to capillaris.

tectorum PARENT	F ₁ ACHENES	GERMINATED	UNCLAS- SIFIED ¹	LIVED	DIED	GENOTYPE OF tectorum PARENT
28H.T. 1498I- 1	21	8			8	l l
" " - 2	16	3			3	
" " - 3	37	5			5	
" " - 4	22	14			14	l l
" " - 5	22	7			7	l l
" " - 6	6	6			6	l l
" " - 7	29	16			16	l l
" " - 8	27	15			15	l l
" " -10	29	26			26	l l
" " -11	26	11			11	l l
" " -13	31	27			27	l l
" " -14	27	25			25	l l
" " -15	20	16	1		15	l l
" " -16	8	7			7	l l
Total	321	186	1		185	

¹ Did not survive transplanting.

Figures 2, 3, and 4 show three flats of young hybrids whose *lectorum* parents were the progeny of plants of the three genotypes. Figure 2 shows hybrids whose various *lectorum* parents were the progeny of *L L* plants—the hybrids are all developing normally. Figure 3 shows hybrids whose *lectorum* parents were the progeny of *L l* plants. Each kind of hybrid population obtained from a segregating group of *lectorum* plants is represented: all living; part living; part living, part dying; all dying.

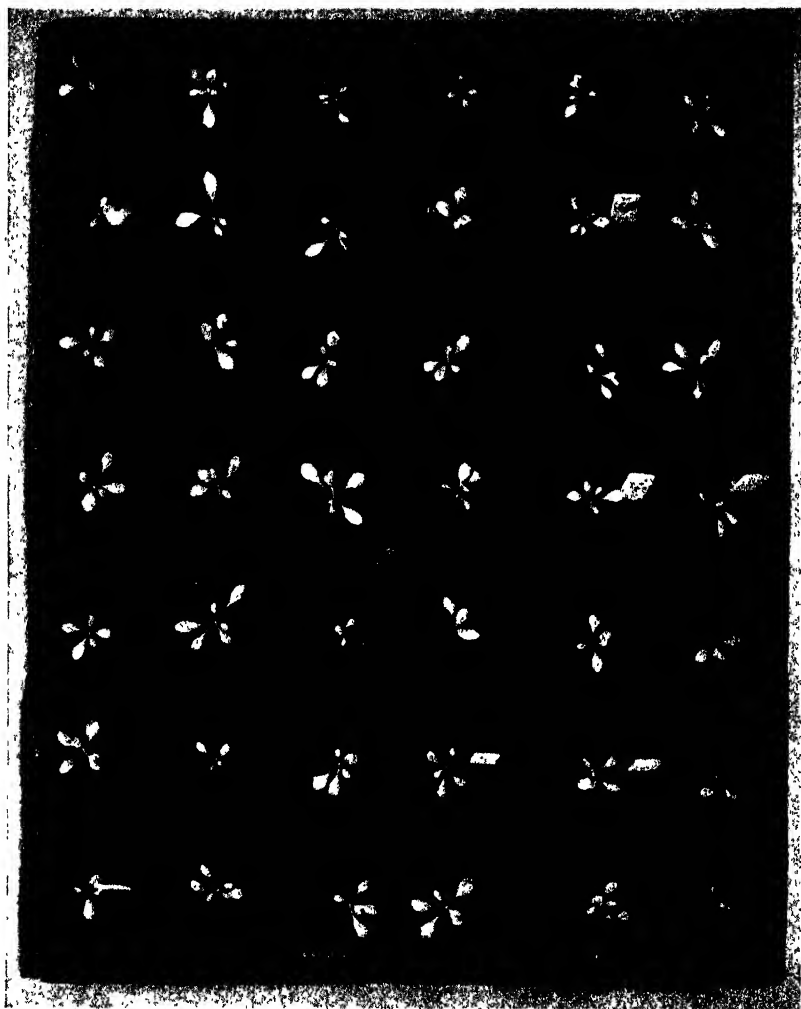


FIGURE 2.—*C. capillaris-lectorum* hybrids whose *lectorum* parents were *LL*. A label marks the first plant of a population numbering from below in each row.

Figure 4 shows hybrids whose *tectorum* parents were the progeny of *ll* plants—all are dying at the cotyledon stage.

Table 10 summarizes the distribution of the three genotypes in the original segregating population and in the two just described. The agreement between the total actual and the expected is very close.

It was stated earlier that the *capillaris* parent of a hybrid, so far as has been determined, had no effect on the expression of the lethal. Table 11 shows the nature of eight populations of hybrids classified with respect

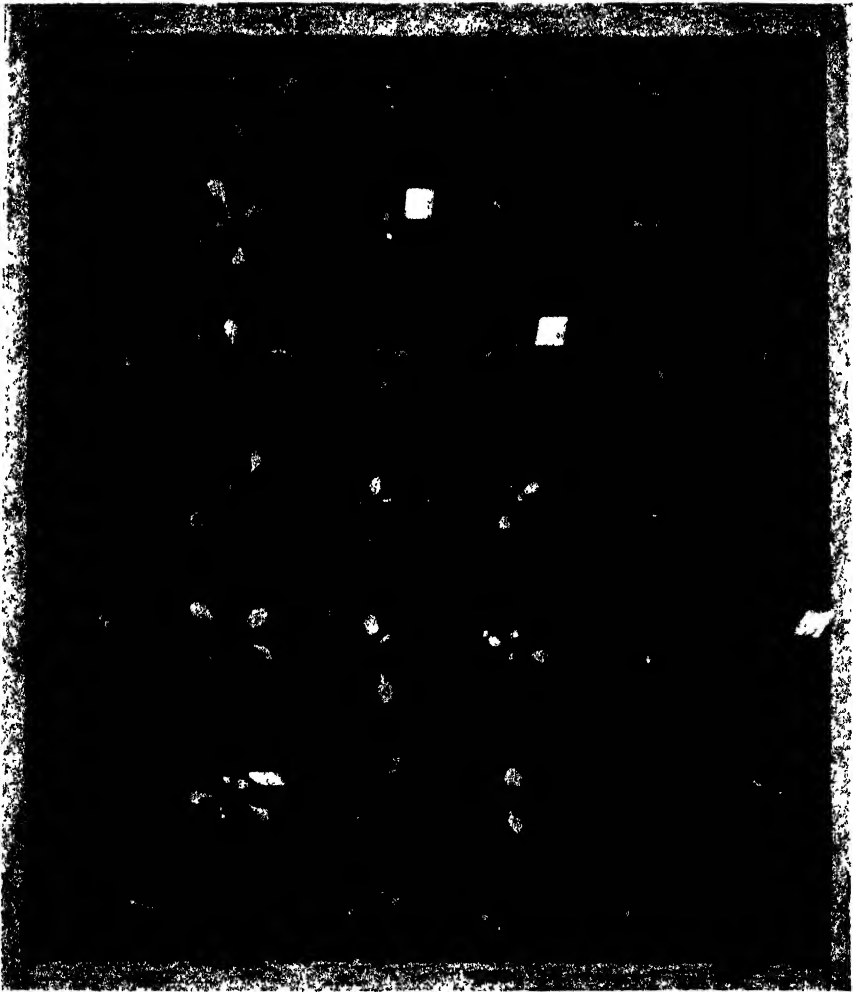


FIGURE 3.—*C. capillaris-tectorum* hybrids whose *tectorum* parents were *ll*.

TABLE 10
Distribution of tectorum plants in the three segregating populations investigated.

HETEROZYGOUS <i>tectorum</i> PARENT	SEGREGATING POPULATION	<i>LL</i>	<i>Ll</i>	<i>ll</i>
T. 23. 1498-4	(T. 26. 1498-1 to 91 (T. 27. 1498-21 to 40	10	13	8
T. 27. 1498-30	28H. T. 1498F-1 to 24	6	11	7
T. 27. 1498-34	28H T 1498G-1 to 32	4	19	4
	Total	20	43	19
	<i>Expected</i>	<i>20.5</i>	<i>41</i>	<i>20.5</i>

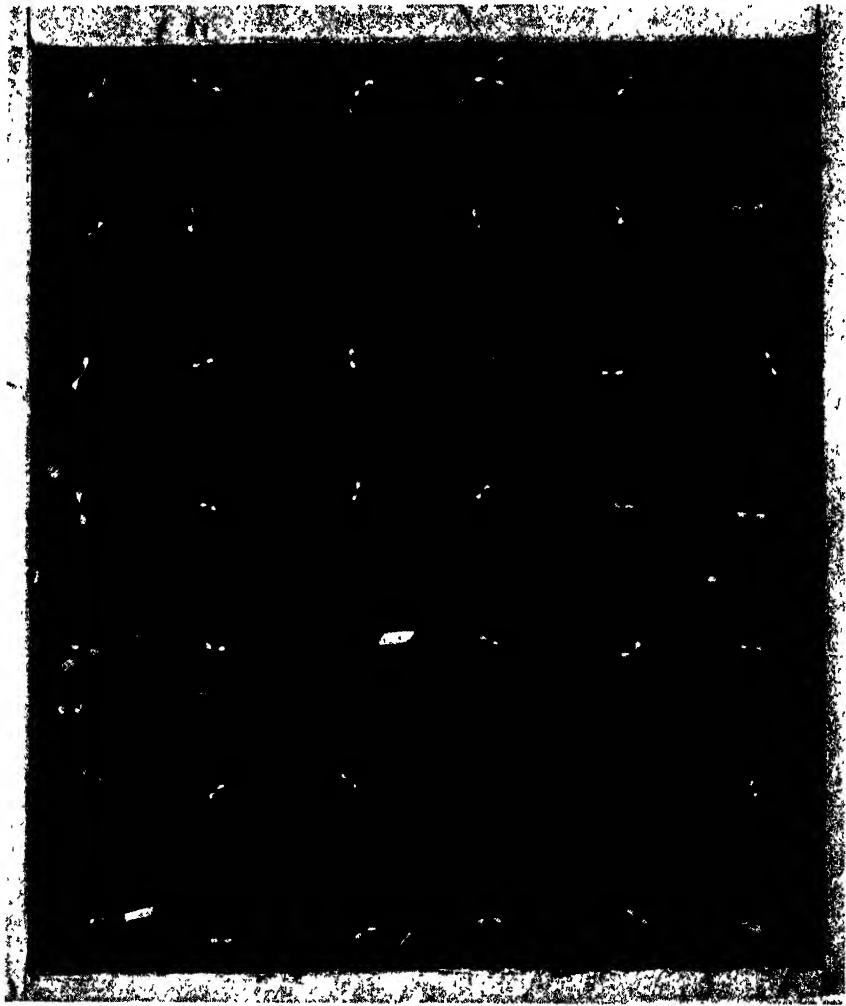


FIGURE 4.—*C. capillaris-tectorum* hybrids whose *tectorum* parents were *ll*.

to their *capillaris* parents. An inspection of the table shows that the various hybrid groups obtained from different *capillaris* parents and the

TABLE 11
Eight hybrid populations classified with respect to their capillaris parents.

<i>tectorum</i> PARENT	<i>capillaris</i> PARENT	HYBRIDS LIVED	HYBRIDS DIED
T. 26. 1648-3	Crane 26-1	2	0
	X 26-2	8	0
T. 26. 1498-68	D 26-8	0	1
	X 27-3	0	2
	X 27H. 8-3	0	7
	X 27H. 21-4	0	15
T. 26. 1498-91	D 26-8	2	0
	X 27-4	2	0
	X 27H. 21-4	7	0
T. 27. 1498-22	Crane 27H. 9-6	6	0
	X 27H. 20-6	26	0
T. 27. 1498-36	Crane 27H. 9-6	0	7
	X 27-4	0	3
	X 27H. 20-6	0	3
	X 27H. 8-11	0	10
T. 27. 1498-37	Crane 27H. 9-6	4	8
	X 27H. 8-5	8	3
	X 27H. 8-3	3	4
T. 27. 1498	D 26-2	0	1
	X 27-3	0	5
	X 27H. 20-6	0	11
	X 27H. 21-4	0	29
T. 27. 1498-34	D 26-3	2	0
	D 26-8	0	1
	X 27H. 20-6	6	7
	X 27H. 8-7	4	8
	X 27-3	0	2

same *tectorum* parent behave similarly with respect to the lethal. There were three strains of *capillaris* involved. The origin of the X strain has been described. *Capillaris* D is an inbred strain from material collected for Professor J. L. COLLINS near Eureka, California. The Crane strain is from a derivative of a cross between D and another inbred strain from the same

locality. The Crane and D strains have less vigorous habit and mature later than X and the Crane strain was segregating for the "chlorina" character described by COLLINS (1924).

It was expected on the basis of a simple lethal factor that the hybrid progeny of heterozygous (*L l*) *tectorum* plants would give a 1:1 ratio of living to dying individuals. In no case, except the one attributed to experimental error (progeny of T.27.1498-40, table 1), was a distribution found which could not be harmonized with such a ratio. In many cases a good 1:1 ratio was obtained in a single population; in many others the number was too small to give any significance to the distribution. On totalling all the populations from *L l* *tectorum* parents the distribution was found to be 346 living to 339 dying, a good 1:1 ratio.

The percentage of germination of the hybrid achenes is of some interest. As may be seen from the tables wide variations occurred. Table 12 shows,

TABLE 12
Percentage of germination of capillaris-tectorum (1498) hybrids grown in the spring and fall of 1928.

	SPRING 1928			FALL 1928			TOTAL		
	ACHENES	GERMINATED	PERCENTAGE	ACHENES	GERMINATED	PERCENTAGE	ACHENES	GERMINATED	PERCENTAGE
Populations from <i>L L</i> parents	1063	572	53.81	822	538	65.45	1885	1110	58.88
Populations from <i>ll</i> parents	326	206	63.19	773	461	59.64	1099	667	60.69
Populations from <i>L l</i> parents	431	295	68.44	729	374	51.30	1160	669	57.67
Total	1820	1073	58.96	2324	1373	59.08	4144	2446	59.02

however, that for large numbers the percentage is rather constant. The data include all the hybrids between *tectorum* 1498 and *capillaris* made in the summers of 1927 and 1928 and grown in the spring and fall of 1928 respectively (omitting 3 populations in table 6 whose seed pots were upset and an unknown number of achenes lost). The average percentage amounts to only 59.02 percent. There is no consistent difference either between the two crops or between hybrid progenies of parents of different genotypes. These data show that there is no differential germination of hybrids with respect to the lethal and this conclusion is confirmed by the 1:1 ratio of living to dying hybrids from heterozygous parents.

The inviable hybrids obtained by BABCOCK and COLLINS in only a few cases developed tiny first leaves; usually development ceased before any trace of leaves was to be seen. This condition characterized most of the inviable hybrids which were obtained in this investigation but some developed further. Figure 5 is a picture of a normal hybrid and six inviable ones

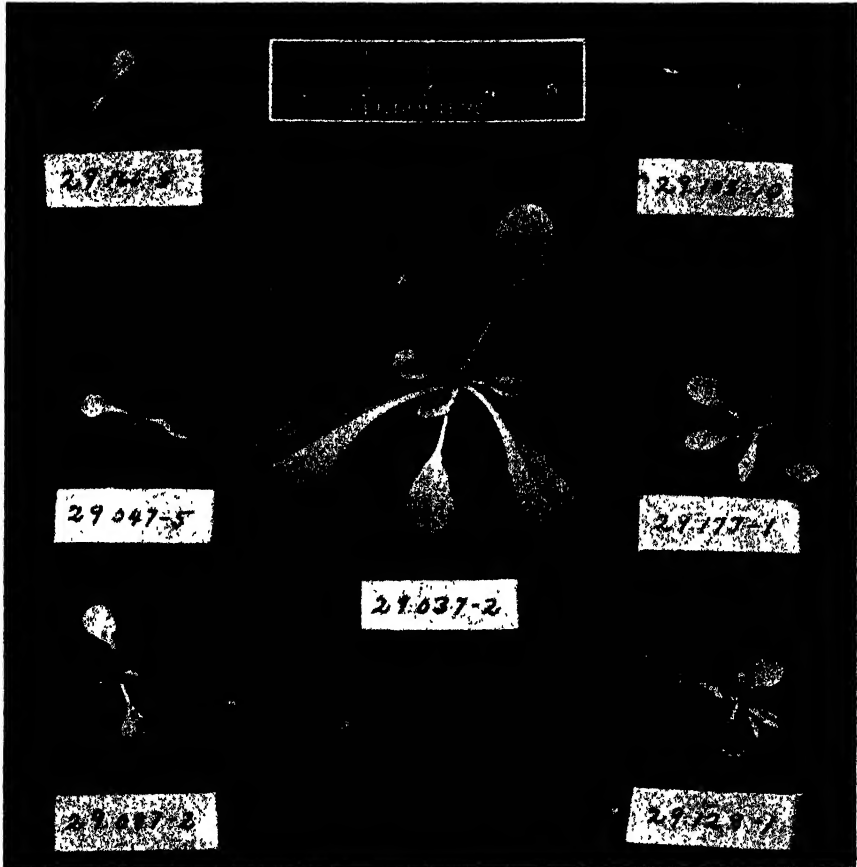


FIGURE 5.—A viable F_1 *C capillaris-lectorum* F_1 hybrid (centre) and six inviable ones chosen to illustrate various degrees of development to which inviable hybrids attained.

transplanted into a small box from their positions in the flats. The inviable ones were chosen to illustrate the various degrees of development to which the hybrids attained. Figure 6 is a picture of the same box of hybrids a month later. In the interval the first four inviable ones died without developing further, but two continued to produce small granular leaves, darker

green in color and coarser in texture than normal leaves. They were showing signs of coming death when the picture was taken. In only a few cases did the inviable hybrids develop to a stage comparable with these last two.

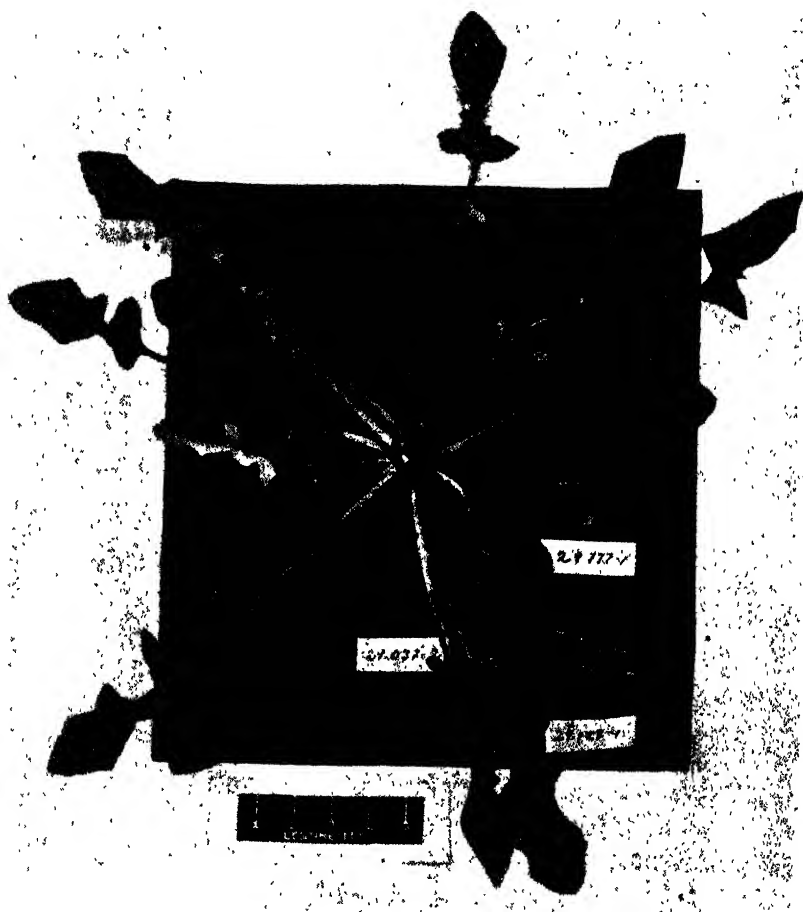


FIGURE 6.—The same box of hybrids a month later.

Three hybrids which had ceased development at the cotyledon stage and two which were obviously abnormal but which had produced a number of leaves were fixed and sectioned for anatomical examination. Normally developing hybrid seedlings of the same age were examined for

comparison. The most striking feature of the inviable hybrids was the occurrence of masses of disintegrating cells which appeared like black slime. This condition was especially characteristic of those seedlings which had ceased development early, being very prevalent in the region just back of the plumule and around the vascular system of the stem and cotyledons. While it was much less prevalent in abnormal hybrids which produced a number of leaves it was more noticeable in them than in normal hybrid seedlings in which there was little or no disintegration. All of the inviable hybrids showed disorganized parenchymatous tissue, the cells being irregular in arrangement and exhibiting striking variations in size and shape which in some cases were probably due to the collapse of intervening cell walls. The plumules of the inviable hybrids which produced no leaves showed several buds in various stages of early development, most of the tissues being still meristematic. The beginnings of differentiation were to be seen in the larger size and vacuolated appearance of some of the cells and in the occurrence of strands of elongated meristematic cells extending upwards through the undeveloped buds. In one plant tracheids were observed extending to within two cell layers of the surface of a bud. These tracheids were much shorter than the recently differentiated tracheids of normal hybrids. The occurrence of tracheids in the meristematic tissue of a very small developing bud is quite abnormal.

The inviable hybrids which developed tiny leaves were still growing when fixed and showed a normal growing point and buds in various stages of development. The shape of the young plant was broad and flat in contrast with the narrower and more upstanding normal hybrid. Irregularity in arrangement and size of the cells of the parenchymatous tissue and the occurrence of small areas of dead cells distinguished them from normal hybrids. Particularly interesting was the occurrence of groups of small cells, sometimes meristematic, which in shape, size, and position resembled axillary buds. In no case did they appear to be developing into leaves. No such structures were seen in normal hybrids.

BABCOCK and COLLINS (1920a) described patches of black slime and irregularities in parenchymatous tissue in their inviable hybrids. Patches of embryonic tissue and of tracheary cells were distributed here and there among the larger vegetative cells. While this latter condition was not observed in these hybrids it could hardly be expected that disorganization causing cessation of development would manifest itself always in the same way and it has been shown above that the various inviable hybrids examined differed noticeably in the degree and kind of irregularities.

INVESTIGATIONS INVOLVING OTHER STRAINS OF *TECTORUM*

Concurrently with the investigations just described crosses were made in an attempt to discover how widely distributed was the supposed lethal factor in *tectorum*. Achenes from a number of sources obtained over a period of years were sown and as many plants as possible were tested by crossing to *capillaris*. Table 13 gives the sources of the *tectorum* plants used, and the number and kinds of hybrids obtained from them.

TABLE 13

F₁ hybrids obtained by crossing tectorum plants of various origins to capillaris.

ORIGIN OF <i>tectorum</i> PARENT	<i>tectorum</i> PARENT	F ₁ ACHENES	GERMI- NATED	LIVED	DIED
Original seed from Stockholm Botanical Garden	T. 27. 1066- 1	13	11	0	11
	" " - 2	20	10	0	10
	" " - 3	24	9	0	9
	" " - 4	19	10	0	10
Ditto	T. 27. 1067- 1	21	10	0	10
Original seed? Chelsea Botanical Garden	T. 27. 1503- 1	15	13	0	13
	" " - 2	14	13	0	13
	" " - 5	36	22	0	22
Copenhagen Botanical Garden through Nawaschin.	T. 27. 1622- 2	8	8	0	8
	" " - 3	11	4	4	0
Ditto	T. 27. 1644- 1	12	6	0	6
	" " - 2	22	15	0	15
	" " - 3	7	2	1	1
	" " - 4	14	10	6	4
Original seed from near Tomsk, Siberia.	T. 26. 1648- 3	12	10	10	0
	T. 27. 1648- 4	12	12	6	6
	" " - 9	27	21	12	9
Original seed from near Kiev.	T. 27. 1689- 4	32	23	0	23
Progeny of T. 26. 1700-6 (open), original seed from Insula Oland, Sweden.	T. 27. 1700- 1a	23	3	3	0
	" " - 3a	22	18	9	9
	" " - 5a	31	18	18	0
	" " - 6a	13	3	3	0
	" " - 8a	10	6	0	6
	" " - 12a	10	10	6	4
Original seed from Upsala Botanical Garden through Berlin.	T. 27. 1719- 1	26	15	0	15
Original seed from Stockholm Botanical Garden through Berlin.	T. 27. 1726- 1	22	15	0	15

TABLE 13 (continued)

ORIGIN OF <i>tectorum</i> PARENT	<i>tectorum</i> PARENT	F ₁ ACHENES	GERMI- NATED	LIVED	DIED
Original seed from Stockholm Botanical Garden through Berlin.	T.27.1757- 3	11	4	0	4
Original seed from Copenhagen Botanical Garden through Berlin.	T.27.1758- 1	15	7	3	4
	" " - 2	10	6	0	6
	" " - 5	5	1	1	0
Original seed, unknown, through Berlin.	T.27.1760- 1	23	10	0	10
	" " - 2	28	8	0	8
	" " - 3	12	6	0	6
	" " - 4	29	12	0	12
Original seed from Stockholm Botanical Garden.	T.27.1883- 1	31	14	0	14
	" " - 2	11	7	0	7
	" " - 3	19	6	0	6
	" " - 4	28	12	0	12
	" " - 5	16	11	0	11
	" " - 6	9	3	0	3
	" " - 7	9	6	0	6
	" " - 8	20	15	0	15
	" " - 9	7	4	0	4
	" " -10	15	9	0	9

Most of the *tectorum* plants tested produced only inviable hybrids and would be classified as homozygous for the lethal. Two accessions, similar in appearance, obtained from Doctor NAWASCHIN and originally from COPENHAGEN BOTANICAL GARDEN were segregating for the lethal. Two plants of the 1648 strain from Tomsk, Siberia, were heterozygous. The population, T.27.1700-a, appeared to be segregating for the lethal, but since its parent, T.26.1700-6, was open-pollinated and the strain is highly self-sterile the lethal may have been introduced from another strain. A strain from the COPENHAGEN BOTANICAL GARDEN contained both the lethal and its allelomorph. From these results it may be seen that the plants to which the lethal can be traced definitely came either from the COPENHAGEN BOTANICAL GARDEN and are probably of Western European origin or from Tomsk in Central Siberia.

INVESTIGATIONS INVOLVING HYBRIDS BETWEEN TECTORUM AND OTHER SPECIES

These investigations were designed to determine whether the lethal which killed *capillaris-tectorum* F₁ hybrids would also be effective in hybrids between *tectorum* and other *Crepis* species. Table 14 shows the crosses

made and the results obtained. Part of the data on *tectorum-leontodontoides* crosses was kindly supplied by Miss PRISCILLA AVERY who was working with this cross.

TABLE 14

F₁ hybrids between tectorum and other species to show the effect of the lethal.

<i>tectorum</i> PARENT	F ₁ ACHENES	GERMINATED	UNCLAS- SIFIED	LIVED	DIED
28H.T.1498E-4 (L L) × <i>bursifolia</i>	4	2	..	2	0
28H.T.1498E-8 (L L) × <i>bursifolia</i>	6	3		3	0
28H.T.1498I-2 (I I) × <i>bursifolia</i>	16	3	.	0	3
T.27.1498-22 (L L) × <i>leontodontoides</i>	7	4	..	4	0
T.27.1498-23 (L I) × <i>leontodontoides</i>	15	10	¹	6	3
28H.T.1498I-2 (I I) × <i>leontodontoides</i>	28	13	..	0	13
28H.T.1498H-15 (I I) × <i>leontodontoides</i>	4	4	.	0	4
T.27.1498-9 (L L) × <i>setosa</i>	13	10	.	10	0
T.27.1498-18 (L L) × <i>setosa</i>	22	10		10	0
T.27.1498-29 (L I) × <i>setosa</i>	9	7		7	0
28H.T.1498D-18 (L L) × <i>taraxacifolia</i>	3	2	.	2	0
28H.T.1498E-8 (L L) × <i>taraxacifolia</i>	1	1		1	0
28H.T.1498E-14 (L L) × <i>taraxacifolia</i>	4	4	.	4	0
28H.T.1498F-4 (L I) × <i>taraxacifolia</i>	9	8		8	0
T.27.1498-23 (L I) × <i>taraxacifolia</i>	6	3		3	0
28H.T.1498H-2 (I I) × <i>taraxacifolia</i>	3	2		2	0
28H.T.1498H-9 (I I) × <i>taraxacifolia</i>	3	2		2	0
28H.T.1498H-19 (I I) × <i>taraxacifolia</i>	2	2	.	1	1

¹ Died early.

The table shows that both viable and inviable hybrids resulted from *tectorum-bursifolia* and *tectorum-leontodontoides* crosses and that they occurred in the populations and in the proportions expected if the lethal were effective. The data on *tectorum-setosa* are not quite conclusive but indicate that the factor is ineffective here since seven viable hybrids were obtained from a *tectorum* plant heterozygous for the lethal and the chance of obtaining such a distribution if the lethal were effective is only 1 in 128.

The data on *tectorum-taraxacifolia* indicate the general ineffectiveness of the lethal in these hybrids. A heterozygous *tectorum* gave eight hybrids, all viable, and the chance of this occurring if the lethal were effective is 1 in 256. Four viable hybrids were obtained from *tectorum* parents homozygous for the lethal but from another one, one viable and one typically inviable hybrid were obtained. Whether the lethal occasionally manifests itself or whether this plant died from other causes has not been determined.

DISCUSSION

This is the first reported instance, so far as the writer knows, of a lethal factor which is effective only in an interspecific hybrid. The phenomenon described by SAX (1921) in hybrids between varieties of wheat resembles the one described here more closely than any other with which the writer is familiar. He describes hybrids between Bluestem and Amby, varieties of *Triticum vulgare*, which produced abnormal hybrids while these varieties crossed with other members of the *vulgare* group gave F_1 plants, fully fertile. The abnormal hybrids between these varieties "appeared to develop normally until they reached a height of 15 to 20 cm. They then ceased to increase in height although they were vigorous and stooled excessively. Often as many as 40 or 50 primary culms were formed but in no case did a true culm develop. The plants were observed for five months but did not develop further." In this case the cross was only a varietal one and the plants in question developed further than the inviable interspecific hybrids in this study. SAX suggested that the case could be analyzed by crossing the F_1 of Amby \times Marquis with Bluestem. If a factor similar to the lethal in *Crepis tectorum* were involved, either this cross or the F_1 of Bluestem \times Marquis crossed with Amby should reveal it.

In *Nicotiana* it has been shown by CLAUSEN (BABCOCK and CLAUSEN 1927) that different varieties of a species may give different results with respect to the vigor of hybrids with another species. A series of varieties of *N. Tabacum* were crossed with two varieties of *N. glauca*, one derived from a California source, the other from Argentine. "With the California variety of *N. glauca* the hybrids were uniformly weak with yellowish leaves short internodes, and a total growth not much greater than 2 feet. With the Argentine variety they were exceptionally vigorous with large dark leaves, long internodes and a height ranging from 6 to 12 feet according to the variety of *N. Tabacum* used." It would be of interest to know whether this difference in the F_1 hybrids could be ascribed to simple genetic differences between the varieties of *N. glauca*. Such results warn the investigator against drawing conclusions concerning taxonomic relationship from the viability of interspecific hybrids.

From an evolutionary standpoint there is no evidence as to whether this *Crepis* lethal or its allelomorph is the older. Both were found in Western European and Central Siberian strains, indicating a wide range for each. Since the lethal has no effect on the parental species, and since normal F_1 interspecific hybrids are almost sterile it is difficult to see how it has had any part in the later evolutionary development of the species in-

vestigated. If the lethal is the older its present existence may be a relic from a time when the species concerned were in the process of differentiation and it may have played a rôle in the maintenance of the ancestral stocks.

The nature of the causes of interspecific incompatibility has long been a matter of considerable interest and speculation. Genetic investigations are slowly bringing to light conditions which may constitute these causes. That crossability depends upon the particular parental variety used has been shown by BACKHOUSE (1916) and by GAINES and STEVENSON (1922) in wheat-rye crosses and later investigators (THOMPSON 1926, LEIGHTY and SANDO 1928) have confirmed this knowledge. The investigations of BACKHOUSE showed that the difference between the varieties of wheat in their ability to cross readily with rye might rest on a factor basis. Here the incompatibility between the parental species manifests itself in failure of fertilization or possibly early development but when that has been accomplished the hybrid grows vigorously. The existence of the lethal factor which operates to kill an interspecific hybrid at an early stage in development and the demonstration of its heritability in a simple Mendelian fashion is a point of importance in this connection.

As was pointed out above the designations *l* and *L* for the lethal and its allelomorph are not intended to indicate any dominant or recessive relationship. The fact that the lethal has no effect when present doubly in *tectorum* and that it exhibits its characteristic effect when present singly in the hybrid precludes any decision as to which, if either, is dominant.

Any discussion of the peculiar mode of behavior of this lethal factor must be purely speculative. Its lethal effect appears to be the result of failure of development at a stage when differentiation is the characteristic process. The ineffectiveness of the lethal in *tectorum* might indicate that this failure to develop normally involves some relationship between the gene complexes of the parental species. It has been shown (HOLLINGSHEAD 1928) that a plant will develop in a normal manner with a haploid complex of *capillaris* and it is likely that the same would apply to *tectorum*. It was suggested by BABCOCK (1924) that the mode of development determined by the haploid complex of the one species was incompatible with that determined by the haploid complex of the other. This hypothesis, however, does not explain why there is no apparent incompatibility between the mode of development determined by the *tectorum* complex containing the lethal allelomorph and that determined by the *capillaris* complex, nor between the modes of development of the *tectorum* complexes containing respectively the lethal and its allelomorph. If such an explanation was

the correct one it would mean that the mode of development determined by the *tectorum* complex containing the lethal allelomorph is compatible with either that determined by the *capillaris* complex or by the *tectorum* complex containing the lethal but that the last two are incompatible with each other.

The ineffectiveness of the lethal in *tectorum* might be explained by assuming an inhibitor. The lethal effect in the hybrid could be accounted for by assuming two genes in *capillaris* corresponding to those in *tectorum*, one recessive to the lethal and one dominant to the inhibitor. While without experimental foundation such an explanation is not inconceivable in view of the fact that GOULDEN (1925) and THOMPSON (1928) have found factors inhibiting the effect of dwarfing genes in wheat, and corresponding genes in two species of *Drosophila* have been established (MORGAN, BRIDGES and STURTEVANT 1925). Such an explanation would involve the assumption of the same or similar corresponding genes in the two species (*setosa* and *taraxacifolia*) which give hybrids unaffected by the lethal.

SUMMARY

Interspecific hybrids of *Crepis capillaris* and *C. tectorum* previously investigated developed only to the cotyledon stage. In renewed attempts to secure viable hybrids using different strains of the parental species one hybrid population was obtained of which half matured normally and half were inviable. Investigation showed that the *tectorum* parent had been heterozygous for a lethal factor which halted the development of the hybrid to which it was transmitted at the cotyledon stage. Progeny of the *tectorum* plant were all normal but on crossing the various plants to *capillaris* it was evident that the progeny were segregating for the lethal in a 1 : 2 : 1 ratio. Plants homozygous for the lethal allelomorph (LL) gave only viable hybrids, those homozygous for the lethal (ll) gave only inviable hybrids, and those heterozygous for it (Ll) gave hybrids half viable, half inviable. Progenies from plants of each genotype were grown and their constitution tested by crossing with *capillaris*. All the progeny of LL proved to be LL giving viable hybrids only. Progeny of Ll segregated in a 1 LL : 2 Ll : 1 ll ratio giving hybrid populations of three kinds: (1) all viable, (2) half viable, half inviable, (3) all inviable. All the progeny of ll were ll giving only inviable hybrids.

The viability of the hybrids was independent of the *capillaris* parent used and there was no differential germination with respect to the lethal.

The lethal and its allelomorph have been found in *tectorum* strains from two widely separated localities.

There is evidence that the same lethal is effective in hybrids between *tectorum* and two other *Crepis* species, but hybrids with a third and fourth are unaffected by it.

The lethal may have played a rôle in maintaining ancestral stocks during the early differentiation of the species.

No experimental evidence is available to explain the ineffectiveness of the lethal in the parental species and its effect on an interspecific hybrid but an explanation is proposed which involves an inhibitor of the lethal and corresponding genes in the parental species.

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PROOF THAT THE ENTIRE CHROMOSOME IS NOT ELIMINATED IN THE PRODUCTION OF SOMATIC VARIATIONS BY X-RAYS IN DROSOPHILA

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I have elsewhere pointed out (1928, 1929a, 1929b) that two effects are produced by X-rays in the somatic cells of *Drosophila melanogaster*. One effect is the production of gene mutations, the other, that of breakages in the chromosomes. The effects are both revealed as variant areas in the soma, and such areas are usually identical in appearance whether they are due to breaks or to gene mutations. The chief difference is, in the case of sex-linked characters, that the variant areas in males are the result of gene mutations alone, while those appearing in the females (heterozygous for a sex-linked factor) may result from either cause.

This point may be made clear by citing a single experiment. If normal red-eyed females are crossed to white-eyed mutant males, and their larval offspring X-rayed, a certain proportion of the F_1 flies will show white variant areas or patches on their eyes. Each variant area is composed of a definite number of white ommatidia, and will be large or small, depending on whether early or late stages are treated. This means that if a mutation or breakage is induced in an early somatic cell, the descendant cells will produce an area showing the modification. The number of such areas in the males is found to be only about one-tenth as great as those found among the heterozygous females. Since, in such a cross, the male cell carries the gene for the dominant color factor in the single χ -chromosome present, and since the loss of this factor by breakage of this chromosome would result probably in the death of the cell, a white area on the eye of the male is interpreted as being the result of a gene mutation. The female cell has two χ -chromosomes, and, in the heterozygous condition, one of these carries the dominant color factor and the other carries the recessive factor. If a break occurs in the red-bearing chromosome, and the broken off piece with the dominant factor is lost, a white variant area will be produced, because of the presence of the gene for white in the homologous chromosome. If gene mutations occur in male cells, they must occur with equal frequency in female cells. The excess of white areas found among

the heterozygous females must therefore be due to breakages, or to the elimination of the entire red-bearing χ -chromosome.

An extensive series of experiments has been performed the results of which support the interpretation just given, but up to the present it has not been possible to demonstrate experimentally and conclusively that only a piece and not the whole chromosome is eliminated. Before presenting what may be regarded as a crucial experiment, we shall give such other evidence as seems to support our conclusion—that only a part of the chromosome is lost.

THE EVIDENCE FROM TRANSLOCATIONS

Much evidence, both genetic and cytological, has been obtained by MULLER and PAINTER (1929a, 1929b) showing that when a piece of the chromosome has been broken off by irradiating germ cells it may become attached to another non-homologous chromosome. Such cases are called "translocations." MULLER and PAINTER have been able to detect the attached piece, as well as the remainder of the chromosome from which the piece was broken off. They have worked out a number of such cases and have found that any one of the large chromosomes can be broken by X-rays, resulting in the formation of various kinds of translocations. In somatic cells some broken off pieces could become lost, either in the cytoplasm, or in the case of a translocation or duplication occurring in chromosome halves at a stage when all chromosomes are split, by passing into a sister cell. When a break occurs in a sex chromosome and a piece is lost in the cytoplasm the left-hand piece must be the one that is lost, for it is known that the mantle fiber is attached to the end conventionally represented on the right in the chromosome map. The term break, as used in this paper, carries with it the implication that the broken-off piece is lost, either in the cytoplasm, or through a fractional translocation.

THE EVIDENCE FROM THE EOSIN TEST

In connection with a series of experiments on the mutant eosin the results obtained were of such a nature that some of the variant areas must have followed breaks in the χ -chromosome (PATTERSON 1929a). One line of evidence (admittedly of an unsatisfactory and inconclusive nature) found in this series appeared in the variant areas which follow the loss of the gene for white in the somatic cells of females heterozygous for eosin and white. The eye color of the heterozygous eosin female is a light-eosin, like that of the eosin male, and not dark-eosin as in the homozygous female. The variant areas found among the heterozygous females were of

two kinds, white and dark-eosin. The white areas are due to the loss of the eosin factor or to gene mutations, and the dark-eosin areas were interpreted as probably being due to the loss of the gene for white by breakage. If the entire white-bearing chromosome had been eliminated, then the resultant area would have been light-eosin, and its detection would have been impossible on a light-eosin background. We have assumed that the loss of the gene for white through breakage upsets the previous genic balance between the two χ -chromosomes, with the result that the remaining portion of the broken chromosome so interacts with the factor for eosin in the other chromosome as to produce a dark-eosin area (PATTERSON 1929a). On the other hand, it is also possible that the dark-eosin areas arose through non-disjunction of the halves of an eosin-bearing chromosome, with the resultant formation of a cell containing three χ 's, two bearing eosin and one bearing white; the sister cells would then have contained only one χ , bearing white.

More decisive evidence was found on examination of the results of ray-ing larvae from pure eosin stock. Here it was found that no more light-eosin areas occurred on the eyes of the females than were to be expected as a result of gene mutations. If the cases of chromatin loss had involved the entire chromosome here, we should have observed a relatively great number of light-eosin areas due to this cause, since the eosin eye color produced by a single χ (as in the male) is known to be lighter than that produced by two χ 's (as in the homozygous female). The absence of any light-eosin areas, except those due to gene mutations, thus shows that the cases of chromatin loss must have involved only a section of the chromosome, that is, breakage, and that a cell with a broken chromosome, lacking the gene for eosin, and one whole chromosome containing eosin, produces as dark a color as a cell with two whole eosin-bearing chromosomes.

THE EVIDENCE FROM THE WHITE-LOZENGE TEST

One experiment which further demonstrated that at least some of the white areas were the result of breaks in the χ -chromosome was the white-lozenge test. In this experiment normal red eyed females were crossed to white-lozenge males. Lozenge is also a sex-linked eye character, and its gene is located in the same chromosome as the gene for white. The locus of the gene for lozenge is at locus 27.7 on the chromosome map; it is therefore located 26.2 units to the right of the locus for white (which is at point 1.5). A total of 724 heterozygous females were obtained from treated larvae. It was calculated that among all the white areas found, thirty white-non-lozenge and 129 white-lozenge areas were due to breakage or to the

elimination of the entire red-bearing chromosome. But the thirty cases of white non-lozenge areas must have been produced by a break in the chromosome at some point between the locus for the dominant color factor and the locus for the normal allelomorph of lozenge; otherwise the lozenge character would have appeared in these thirty areas. In the case of the 129 white-lozenge areas, evidently the break occurred to the right of point 27.7, or else the entire red-bearing chromosome was lost.

While the above mentioned tests furnished conclusive evidence that breakage was the cause of many of the variant areas appearing in the females, yet there still remained the possibility that others of them might be due to the loss of the entire chromosome, and realizing this we made several further attempts to settle the question. One of these may be mentioned. Any crucial test would require the use of a sex-linked eye character with its gene location at or near the right-hand end of the chromosome. It just so happened that we had a new mutant eye color, called "carnation" (produced by raying larvae), the gene for which is at point 65.5. This is close to but not at the extreme right-hand end. In making tests with this character it was soon found that "carnation areas" could not be detected with certainty, because the carnation color is almost as deep as that of the normal red. At this point Doctor MULLER suggested that I try one of his "duplication" stocks. The results from this test are given in the next section.

THE THETA TEST

The duplication stock in question is known as " χ -duplication I," but we shall call it *Theta*. It has attached to the extreme right-hand end of the sex-chromosomes, in fact, beyond the mantle fiber—as PAINTER's cytological preparations have demonstrated—a small piece carrying the factor for gray body color, derived from the left-hand end of another sex chromosome. The stock is maintained by crossing heterozygous *Theta* females to yellow red-eyed males. This is done because the homozygous *Theta* females are very infertile. The composition of the sex-chromosomes of the *Theta* females is shown in diagram "A" of the accompanying figure. One of the χ -chromosomes (χ') has, in addition to the factors for yellow body color and red eyes, both of which are located at the left-hand end, the dominant allelomorph for body color, "gray," located at the extreme right-hand end in the small attached fragment. The other χ -chromosome (χ) is identical to its homologue, except that it does not have the extra fragment. Such a female has red eyes and a gray body, and when mated to, yellow red male will give *Theta* females, like their mother, yellow red males, yellow red females, and some *Theta* males.

In making the test for breakage, *Theta* females were crossed to yellow white singed males (instead of to yellow red males), and the early larval stages X-rayed. The composition of the sex-chromosomes of the *Theta* females found in the F_1 generation is shown in diagram B. The *Theta* females have one chromosome with the duplication (χ'), as before, but the other sex-chromosome carries yellow, white and singed (χ). The second class of females, or yellow red, has as before yellow and red in one χ -chromosome, and the yellow-white singed combination in the other χ -chromosome (figure 1, diagram E).

The test was made by treating larval stages and then examining the F_1 females for the different kinds of variant areas. The tabulated results are shown in table 1. There were 188 *Theta* females and 260 yellow females. There were fifteen gray-singed areas, fourteen white areas, four yellow

TABLE 1

Variant areas in females treated in larval stages, from a cross between Theta females and yellow white singed males.

CULTURE	AGE	DOSE	THETA FEMALES				YELLOW FEMALES		
			NUMBER	GRAY-SIN AREAS	WHITE AREAS	YELLOW AREAS	NUMBER	YEL-SIN AREAS	WHITE AREAS
A	7-19	D-3	7	1	0	0	13	0	0
B	8-20	D-3	13	2	0	1	12	1	0
C	9-21	D-3	3	0	0	0	12	0	0
D	12-24	D-3	19	2	1	0	16	0	2
E	18-30	D-3	5	0	2	0	21	1	2
F	19-31	D-4	48	2	2	1	48	1	9
G	24-36	D-3	72	7	8	2	98	1	8
H	24-36	D-4	18	0	1	0	29	1	1
I	31-43	D-3	3	1	0	0	11	0	1
Totals			188	15	14	4	260	5	23
Controls			176	0	0	0	262	0	0

(non-singed) areas, and no yellow-singed areas found among the *Theta* females, and five yellow-singed areas and twenty-three white areas found

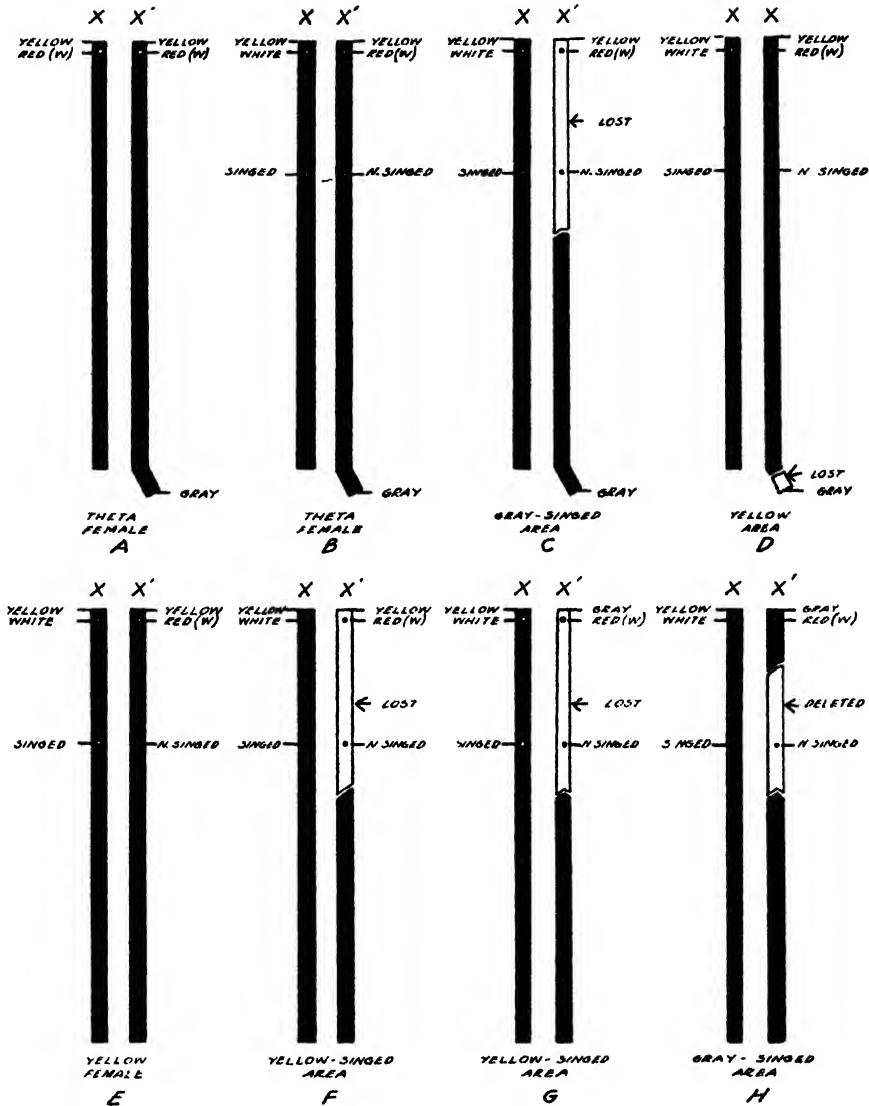


FIGURE 1.—Diagrams showing the composition of the pair of homologous sex chromosomes in cells of females used in the experiments. χ is the chromosome from the father and χ' is the sex chromosome from the mother. A, heterozygous *Theta* female of stock flies. B, heterozygous *Theta* female used in experiment. C, Gray-singed area of *Theta* female. D, yellow area of *Theta* female. E, heterozygous yellow female. F, yellow-singed area of yellow female. G, yellow-singed area of heterozygous wild-type female. H, gray-singed area of heterozygous wild-type female. The latter is a case of deletion. The attached fragment, as represented in diagrams A to D, is not drawn to the same scale as the rest of the chromosome. The main part of the chromosome is drawn as shown in the genetic maps, whereas the fragment is shown as proportionately larger, although genetically it is very small (see figure 8, MULLER and PAINTER 1929a).

among the yellow females. The white areas on the eyes in both types of females are similar to those previously described, and will not be considered further in this paper. The yellow, yellow-singed, and gray-singed areas are found on the body of the fly, and include bristles and hairs. It is these areas that are of interest in this connection.

We may now point out the meaning of these particular types of areas. A gray-singed area on a *Theta* female means that the χ -chromosome carrying the attached fragment was broken at some point to the right of the locus of the gene for the normal allelomorph for singed bristles (figure 1, diagram C). The fact that the singed areas are gray shows that the extra attached fragment is present. If they had been yellow, instead of gray, it would have indicated that the entire chromosome had been lost. All of the fifteen singed areas found among the *Theta* females were gray, showing that in no case was the whole chromosome lost. This test furnishes indisputable evidence in favor of our conclusion, namely, that the variant areas produced in somatic tissues by X-rays are in the main the results of breaks in the chromosomes.

The four cases of yellow areas found on the *Theta* females are of interest because they indicate that the small attached fragment, carrying the dominant color factor for gray body, has been broken off and lost (figure 1, diagram D). The reason why the number of these areas is so few, as compared to the number of gray-singed areas, is due to the fact that there is but a short distance in which a break can take place in the fragment. We should therefore expect to find a much greater number of singed areas because of the greater distance over which a break can take place in the main body of the chromosome and still produce such areas.

In addition to the twenty-three white areas found among the yellow females, there were also five yellow-singed areas. The method by which such areas would be produced by breakage is illustrated in diagram F.

Another interesting question that arose in connection with the *Theta* test is whether the left-hand end of the chromosome is always eliminated, rather than a section from the middle of the chromosome. This point may also be determined. The test was made by crossing normal red eyed females to yellow white singed males, and treating the larvae. The results of this test are shown in table 2. The 275 F_1 females gave sixteen white areas, 7 yellow-singed areas, one gray-singed area, and two yellow areas. We are concerned with the two types of singed areas. Seven out of the eight found were yellow. This means that the left-hand end had been lost (figure 1, diagram G). The origin of the single gray-singed area may be explained in the following manner. It may have resulted from a loss of a section of

that part of the χ -chromosome which carries the factor for non-singed (figure 1, diagram H). Such cases are called "deletions," and it is supposed that the two remaining portions of the chromosome fused together at the time the loss occurred, as found by MULLER and PAINTER in the case of germ cell rayings. It is also possible that the gray-bearing fragment became attached to some other chromosome. In that case a break between

TABLE 2

Variant areas in females treated as larvae, from a cross between normal females and yellow singed white males.

CULTURE	AGE	DOSE	FEMALES	WHITE AREAS	YEL.-SINGED AREAS	GRAY-SINGED AREAS	YELLOW AREAS
A	8-20	D-3	15	1	0	0	0
B	20-32	D-3	93	1	4	0	0
C	24-36	D-3	51	3	1	0	1
D	24-36	D-4	101	9	2	1	1
E	31-43	D-4	15	2	0	0	0
Totals			275	16	7	1	2
Controls			226	0	0	0	0

non-singed and the fiber-bearing end of the chromosome need not have occurred, if this entire section became lost, but, as the other evidence in this paper indicates, loss of the fiber-bearing end rarely if ever occurs.

Throughout this paper we have not discussed the possibility that breaks may take place in the sex chromosome which carries the genes for the sex-linked recessive characters (the chromosome marked χ in all of the diagrams). Such breaks must and do occur, but in the experiments given in this paper their effects could not be detected because of the presence of the dominant factors in the homologous chromosome. Experimental proof that both chromosomes break with equal frequency was obtained and presented in connection with our work on the mutant eosin, to which reference was made above.

We have not heretofore referred to the control flies. These numbers are listed in the two tables. As these data show, no variant area was found among any of the control flies.

CONCLUSION

The evidence presented in this paper supports the conclusion that, in addition to the production of gene mutation, X-rays cause breaks in the chromosome in the somatic cells of *Drosophila*. In the case of the somatic cells of females, heterozygous for a sex-linked character, the effect is revealed as a variant area which shows the recessive character only. This is due to the fact that that portion of the χ -chromosome which carries the gene for the dominant factor (or factors if more than one is involved) has been broken off and has become lost. This allows the recessive factor in the homologous chromosome to reveal itself in the soma as a variant area, composed of the descendant cells of the original affected cell. One may not be justified in saying that variant areas are always thus produced, for it may still be argued that the entire chromosome is occasionally lost. However, the "*Theta* test" described above demonstrated that in all cases there observed (19) only a part of the chromosome was lost.

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GAMETOPHYTIC INHERITANCE IN SPHAEROCARPOS.

IV. FURTHER STUDIES OF TUFTEDNESS AND POLYCLADY

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In previous papers dealing with the genetics of *Sphaerocarpos Donnellii* Aust., the possibility has been pointed out of an analysis of inheritance by means of the distribution of genetic tendencies among the persistently adherent spores formed from a single spore mother cell. In work thus far reported the possibility has been realized only in a limited way, largely because of the difficulty of securing germination of all the spores of a tetrad. With reference to 2 vegetative gametophytic characters, as well as sex, a considerable amount of material is now available which, although far from satisfactory in various respects, seems to justify an attempt to use this method of analysis on a larger scale than hitherto. Certain definite conclusions are possible from the results here presented. In considerable measure, however, they are but suggestive of future possibilities.

The work reported in this paper has been carried on with the assistance, at different times, of MR. JAMES A. LOUNSBURY, DOCTOR GEORGE O. COOPER, MISS BEATRICE I. NEVINS, DOCTOR OPHELIA C. WESLEY, and MISS INEVA F. REILLY. This assistance was made possible by grants from the research fund of the UNIVERSITY OF WISCONSIN.

THE TUFTED CHARACTER

In an earlier paper (ALLEN 1924) the occurrence and characteristics of "tufted" clones were discussed in detail. Apart from some aberrancies in form of thallus lobes and in habit of growth, the outstanding peculiarity of a tufted gametophyte is a tendency for its archegonial or antheridial involucre to depart, often to an extreme degree, from the typical form. The character is a variable one; some or many of the branches in a tufted clone are indistinguishable from those of a typical clone; other branches bear both typical and variant involucre, and on still others all the involucre are markedly atypical.

Tufted clones were isolated on a number of occasions from material received from Sanford, Florida. Races of this character are apparently not uncommon, at least in that locality.

The previous study has shown that tuftedness is inherited vegetatively; even a strictly typical branch of a tufted clone gives rise sooner or later to tufted branches. The tufted character is likewise inherited through gametes and spores; that is, tufted and nontufted clones are genotypically different. It has also appeared that between tufted clones there are genetic differ-

ences, indicated by the appearance in different clones of very different proportions of typical and tufted branches. Cases were cited in the previous paper illustrating the fact that, while the same clone may at different times include varying proportions of tufted branches, nevertheless some clones consistently over a term of years present a very high proportion, others a median, and still others a very low, proportion of such branches.

If several factors exist for tuftedness, it might be expected that these factors, present singly, would produce different phenotypic results. Repeated study of numerous tufted clones with this possibility in mind has, however, disclosed no diagnostic differences, save in the degree to which the tufted tendency is expressed.

The method has been described by which the proportion of tuftedness of a given clone is calculated. At each observation, an estimate is made of the proportion of the area of the soil in a three-inch pot covered by the clone; and of the proportion of tufted branches (that is, of branches bearing preponderantly atypical involucre). From the figures so obtained, the proportion, expressed as a percentage, is determined of the number of tufted branches observed to the total number of branches present in the clone at the times of all observations, successive observations having been at least three months apart. Percentages, when given in the present paper, are stated as the nearest whole numbers; in case the calculated percentage is less than 0.5, it is given as "slight" ("sl."). Since the calculation is based upon estimates, and since the actual proportion of tuftedness characterizing a particular clone varies from time to time, probably in response to environmental changes, it is evident that the percentages given must be considered only as rough approximations. With all due allowances, however, they afford some measure of the genetic differences which seem clearly to exist.

The percentages will be noted to range from "slight" to considerably over 90 percent. In no case has a tufted clone which lived for any considerable time failed to produce at least an occasional typical branch. At the opposite extreme are clones which, during a life of several years, have been observed to produce only one or a very few tufted branches. At this extreme, obviously, the distinction between a genetically tufted and a genetically nontufted clone is uncertain, since the former may not have been observed to bear tufted branches. Some tufted clones have, therefore, doubtless been classed as nontufted; and the occurrence of such an error is the more likely the shorter the period of life of such a clone. The opposite error is much less likely but not impossible, because the distinction may not always be certain as between a rare tufted branch and one with

several atypical involucre which still falls within the limits of variability of a nontufted race. This difficulty is illustrated by the classification at first adopted of "possibly atypical" clones. Later work (ALLEN 1926) has shown that some such clones, phenotypically of doubtful character, are genotypically nontufted. The whole picture thus afforded is that of a continuous series (or one of an incalculable number of terms) connecting an extreme of remarkably uniform ("typical") character with the opposite extreme of very high but never quite uniform deviation from the typical condition.

Another source of possible error is the division of a sporeling into 2 before its isolation from its tetrad sibs. The majority of cases of this nature noted were among the offspring of spore tetrads sown in the autumn of 1923. In consequence of my absence from Madison during part of the following winter and the spring, some of these sporelings were separated only after they had attained a relatively large size. The possibility of a previous division was thus of course increased. Even under most favorable circumstances, however, an error of this nature is not excluded.

Such an error is detected if it results in the appearance of more than 2 female, or more than 2 male, clones coming from a single tetrad. In each such case the error was corrected in the tabulation of results, as noted in the discussion of the respective matings. The occurrence of 3 clones of 1 sex might in some instances suggest an actual deviation from the ordinary sex ratio (of 2 females and 2 males from each tetrad). However, none of the cases of this nature noted in the present work seems to be susceptible of such an interpretation; sometimes because the total number of apparently separate sporelings from a tetrad was more than 4; more often because circumstances noted at the time of isolation of certain sporelings cast doubt upon their separate origin. It seems safe therefore to assume that, so far as my work to date is concerned, every tetrad, had all its spores germinated, would have given rise to 2 female and 2 male clones.

A similar error might remain undiscovered if, for example, only one of the genetically female spores of a tetrad were to germinate, the resultant sporeling then becoming divided. Such errors, if, as is possible, they have at times occurred, have not seriously affected the results discussed in the present paper for the reason that, as shown in the tabulations, in the large majority of cases the 2 female, or the 2 male, clones arising from a tetrad are genetically similar. In a minority of cases, as will appear, the distribution of the polycladous—nonpolycladous character pair is distinct from that of the sexual characters, the result being the production of four genetically different gametophytes from a tetrad. Apparently a similar inde-

pendent segregation may occur involving sex and tuftedness. An error of the sort in question might tend to reduce the apparent proportional occurrence of such an intra-tetrad distribution.

In the first paper of this series (ALLEN 1924) the results were compared of matings of nontufted \times nontufted, and of tufted \times nontufted. In the work here reported, the study has been extended to the offspring of nontufted \times tufted, and of tufted \times tufted. The tufted males used as parents were descendants of the tufted females of previous matings. A comparison has been made also between the genetic behavior of little-tufted and much-tufted clones.

Since gametophytic characters only are being considered, the maternal and paternal clones used in the respective matings (the p_1 generation) will usually be referred to as "parents," and their gametophytic descendants of the f_1 generation as "offspring." Strictly speaking, of course, the p_1 gametophytes are the *grandparents* of the f_1 gametophytes, the immediate offspring of the former being F_1 sporophytes.

Nontufted ♀ ♀ \times Much-tufted ♂ ♂

The female clones used in the experiments of this series were all strictly nontufted so far as numerous observations over considerable periods have disclosed. The male clones were selected as showing a large proportion of tuftedness.

The date of fertilization given in each case is that at which portions of the female and male clones in question, after being placed in the same pot, were first flooded with sterilized water. Since the flooding was usually repeated several times at intervals of one to several days, the actual date of fertilization may have been, in some instances, later than that given.

Mating 19. 21.215 (nontufted) \times 21.16 (75 percent tufted)

Fertilized May 12, 1922. Mature sporophytes dried July 7. Twenty spore tetrads from each of 2 sporophytes sown separately October 20 and 24. Sporelings transplanted February 12–March 24, 1923.

The mother in this mating was an offspring of mating 1 (nontufted \times nontufted; ALLEN 1924). At the time of 3 observations during its first year's growth it showed a rather large proportion of irregularities; numerous observations during the succeeding 6 years, however, have shown it to be typical. This was one of the clones which were first classed as "possibly atypical," but later results (ALLEN 1926) showed several such clones to be genetically similar to others classed as "typical."

The father was an offspring of mating 14 (tufted \times polycladous; ALLEN

1925). The structures of this clone were at times, as previously reported, so aberrant as to suggest that it might be genetically polycladous as well as tufted. But, since none of its progeny have shown the polycladous character (in the present mating and in mating 20, reported below), it is clearly to be classed as tufted. The percentage of tuftedness to be ascribed to it has been more than usually doubtful; roughly it is placed at 75 percent.

The results of the present mating as well as those of mating 26 have been provisionally reported (ALLEN 1924a). Such differences as appear in the distribution of the offspring of these 2 families between table 2 of the earlier paper and tables 1 and 3 of the present report result in part from the fact that a few clones earlier classed as typical have later shown themselves to be tufted, and in part from the omission (in the present paper) of a few clones whose determination seemed unsatisfactory in view of their early death. No clone is here classed as nontufted which was not so determined as a result of at least two observations some months apart.

In 1 instance in this family, referred to previously (ALLEN 1924a, p. 228), 2 groups of 2 plants each appeared in 1 pot. In 1 group were 1 nontufted female and 1 plant of undetermined sex; in the other group, 1 nontufted female and 1 female of undetermined character. These were omitted from the previous tabulation; they are included in the present one as representing 2 tetrads, each yielding (so far as determinations were possible) 1 nontufted female. In another pot, 2 distinct groups appeared, each consisting of 2 females and 2 males. It seemed clear that 2 separate spore tetrads were here involved, and the results are so treated. One tetrad, also noted in the earlier paper, apparently gave rise to 1 nontufted female, 1 tufted male, 1 nontufted male, and 1 male of undetermined character. At the time of transplanting, it was noted that the last-named male and the male classed as nontufted were possibly parts of the same plant. That is here considered to have been the case.

One female nontufted clone (23.110) has shown a large proportion of involuclral appendages as well as some minor irregularities in the forms of the involuclres. While appendages occur rarely on the involuclres in many clones that are otherwise typical, a few female clones and at least 1 male clone had previously appeared in which such appendages were characteristically numerous. This character has been briefly discussed (ALLEN 1924, pp. 576, 577). The difficulty of studying its inheritance is increased by the fact that involuclral appendages are frequent in the tufted clones; in a clone with a tufted ancestry like the one under consideration, it is possible, therefore, that the appendages are an expression of the tufted tendency. But since, during 6 years, no tufted branch has been seen in

clone 23.110, it is provisionally classed as an "appendiculate" mutant.

The offspring in this family from the tetrads of two sporophytes are grouped together in table 1. No significant differences have been noted between the offspring of distinct sporophytes derived from the same mating.

Mating 20. 21.116 (nontufted) \times 21.16 (75 percent tufted)

Fertilized May 12, 1922. Sporophytes dried July 12. Ten tetrads from one sporophyte sown November 3. Sporelings transplanted March 29 and April 9, 1923.

The mother in this, like that in the previous mating, was an offspring of mating 1. Numerous observations have determined it without question as nontufted. The father was the same as in mating 19.

Comparatively few spores resulting from this mating germinated (table 1), and some of the f_1 clones died while young. It is probable that the apparently small proportion of tufted offspring is to some extent misleading.

Mating 21. 21.224 (nontufted) \times 20.60 (89 percent tufted)

Fertilized February 10, 1923. Sporophytes dried May 1. Forty-eight tetrads from one sporophyte sown September 18. Sporelings transplanted December 22, 1923–January 27, 1924.

The mother in this case was an offspring of mating 1. It has shown a uniformly typical character. The father was an offspring of mating 8 (tufted, separable \times nontufted; ALLEN 1924). The high percentage of tuftedness assigned it is an average of 19 observations showing respectively from two-thirds to more than nine-tenths tufted branches.

In 5 instances in this family the germinations from a tetrad apparently resulted in more than the 2 expected females. This is 1 of the families with reference to which the records show that some groups of the sporelings were so far advanced in growth when they were transplanted as to make their separation difficult. For reasons already outlined these cases were treated, for purposes of classification, as follows:

(a) Four females, all nontufted; considered as 2 nontufted females. (b) Similar to a. (c) 2 tufted females, 1 nontufted female, 1 tufted male; considered as 1 tufted female, 1 nontufted female, 1 tufted male. (d) 1 tufted, 2 nontufted females; considered as 1 tufted, 1 nontufted. (e) 3 tufted females. At the time of transplanting, the third plant was noted as possibly a fragment of the second; it was so considered in the classification.

With these emendations, the results of this mating are as shown in table 1. The nontufted "appendiculate" female clone (23.5114) occurring in this family is similar to clone 23.110 descended from mating 19.

Mating 22. 21.116 (nontufted) × 20.60 (89 percent tufted)

Fertilized February 24, 1923. Sporophytes dried April 26. Sixty tetrads from one sporophyte sown September 20. Sporelings transplanted January 27–February 1, 1924.

TABLE 1

Results of matings of nontufted × much-tufted; distribution of offspring according to spore tetrads from which they arose (M. = mating; t. = tufted; nt. = nontufted).

P.		M.				TOTALS
♀	♂	M. 19	M. 20	M. 21	M. 22	
(a) 2 nt.	1 t.	2	..	1	1	4
(b) 2 nt.	1	2	2	1	6
(c) 1 nt.	1 t.	1	..	1	..	2
(d) 1 nt.	.	3	3	4	4	14
(e) . .	2 t.	..	.	1	..	1
(f)	1 t.	1	.	2	.	3
						30
(g) 2 t.	2 nt.	3	3
(h) 2 t.	1 nt.	2	.	1	.	3
(i) 2 t.	.. .	7	..	4	1	12
(j) 1 t.	2 nt.	1	1
(k) 1 t.	1 nt.	1	..	1	.	2
(l) 1 t.	1	1	3	2	7
(m) . .	2 nt.	.	..	1	.	1
(n)	1 nt.	..	.	1	2	3
						32
(o) 2 nt.	2 nt.	2	.	1	..	3
(p) 2 nt.	1 nt.	2	2
(q) 1 nt.	2 nt.	.	1	.	1	2
(r) 1 nt.	1 nt.	.	1	..	2§	3
(s) 1 nt., 1 t.	2 nt.	2	2
(t) 1 nt., 1 t.	1 nt.	2	.	.	.	2
(u) 1 nt., 1 t.	.	2	.	3†	2	7
(v) 1 nt.	1 nt., 1 t.	1	.	.	.	1
(w) . . .	1 nt., 1 t.	..	1	.	.	1
						23
(x) 1 nt., 1 t.	1 t.	1*	..	1	1	3
(y) 2 t.	2 t.	1	.	.	.	1
(z) 2 t.	1 t.	..	.	1	.	1
						5
Totals		36	9	28	17	90

Total no. ♀ nontufted clones, 66; ♀ tufted, 64; ♂ nontufted, 41; ♂ tufted, 19. Character not determined: ♀, 3; ♂, 30; sex undetermined, 4. Totals, all sporelings: ♀, 133; ♂, 90; sex unknown, 4; grand total, 227.

* Nontufted ♀ is "appendiculate" (probably a mutant).

† One nontufted ♀ is appendiculate.

§ One nontufted ♂ is a dwarf mutant.

The mother was the same as in mating 20; the father, the same as in mating 21. The results are shown in table 1.

In one instance in this family the offspring from a single spore tetrad consisted of 1 female and 3 males, all nontufted. This group is classed as 1 nontufted female, 2 nontufted males.

One case of an undoubted mutant occurs in this family. It is a male clone (23.6002), substantially resembling a typical male except that all parts, including the involucre, are much smaller. No other similar plant has been observed. In 5 matings with as many different female clones this dwarf male has failed to produce sporophytic offspring.

Like previous studies on *Sphaerocarpos*, the present one gives no indication of a departure from the rule that, if all spores of a tetrad germinate, 2 give rise to female, and 2 to male, gametophytes. The apparent exceptions, as already noted, are explainable, most of them obviously so, as due to an early separation of a single plant into 2.

As pointed out in previous papers, if segregation of chromosomes or of chromosome parts is limited to the heterotypic division in the spore mother cells (and if the distinction between tuftedness and nontuftedness behaves as though due to a single factorial difference) the tetrads in matings like those here considered would be expected to be of 2 classes: those yielding on germination 2 nontufted females, 2 tufted males; and those yielding 2 tufted females, 2 nontufted males. Of the tetrads whose offspring are reported in table 1, those in series a to f inclusive belong or may belong to the former class (allowing for the failure of many of the spores to germinate); and those in series g to n may belong to the latter class. That the tetrads of these two classes are substantially equal (30 : 32) is perhaps significant; for equality would be expected if there is no linkage between tuftedness (or its reverse) and sex.

The 28 tetrads in series o to z inclusive (table 1) do not seem to fall in either of the "expected" classes. With reference to a large proportion of these apparently exceptional tetrads, however, the possibility of an error in classification is to be considered, the error, namely, resulting from the failure of a genetically tufted clone to produce tufted branches. Such an error is especially likely in the case of a clone which died before it had reached a considerable size, and on which but a limited number of observations were made. Among the clones represented in the series in question were many, especially among the males, which died young. So far as concerns series o to w, it is clear that corrections might be made by changing individual clones from the nontufted to the tufted category so that each of

the 23 tetrads included in these series would be referred to one of the two classes represented respectively by series a to f and series g to n. This would reduce the number of apparently exceptional tetrads from 28 to the 5 included in series x, y, and z. That such a correction should be made for many of the male clones is indicated by the discrepancy in total numbers between male nontufted (41) and male tufted clones (19), as compared with the near-equality in number between female nontufted and female tufted clones (66 : 64).

On the other hand, it is not clear that all the tetrads in series o to w should thus be removed from the "exceptional" category. In a few of the apparently "exceptional" cases, the clones, or certain of the clones, classed as tufted have been in existence so long, and have been examined so often, that it seems improbable that, if genetically tufted, they should not have betrayed the fact. While certainty can not be attained, the possibility is not to be dismissed that 3 or even 4 nontufted clones may develop from a spore tetrad in such a family as one of those here considered.

With reference to the possibility of the occurrence of more than 2 tufted clones among the offspring of a single tetrad, illustrated by series y and z, there is less doubt. As already pointed out, while a tufted clone may for a long time produce no tufted branches and may therefore be classed as nontufted, a genetically nontufted clone never produces tufted branches. The 2 cases falling within these series, therefore, in spite of the smallness of the number, indicate that now and then among the offspring of a mating of tufted by nontufted may appear a tetrad 3 or all 4 of whose spores carry the tufted potentiality.

Still another possibility is suggested by series t to x, table 1. This is the development of 2 tufted and 2 nontufted clones from a single tetrad, 1 male and 1 female being tufted and 1 male and 1 female nontufted. A precisely similar distribution of the polycladous and nonpolycladous characters independently of sex, resulting in the production of a small proportion of "four-type" tetrads, has been clearly established (ALLEN 1924a, 1926a), and such distributions will be discussed on a later page. With reference to tuftedness the evidence is less conclusive, because of the ever-present possibility of the error of determining a tufted clone as nontufted. However, in view of the very confident determinations of the characters of some of the clone tetrads concerned, as well as of the certain recognition of a corresponding distribution in the case of the polycladous character, it is concluded provisionally that in a minority of cases a spore tetrad may give rise to 1 nontufted and 1 tufted female, 1 nontufted and 1 tufted male.

Little-tufted ♀ ♀ × *Little-tufted* ♂ ♂

Mating 23. 20.29 (8 percent tufted) × 20.103 (9 percent tufted)

Fertilized February 12, 1923. Sporophytes dried June 26. Spores (separate) from 1 sporophyte sown September 23. Sporelings transplanted December 5, 1923–January 26, 1924.

Both parents used in this mating were among the offspring of mating 8 (ALLEN 1924). The mother in mating 8 was the clone (R27E) whose sporophytic offspring were the first observed to produce separate spores (ALLEN 1925a). Since the spore-separation tendency is transmitted through all the female descendants of clone R27E, the sporophytes resulting from mating 23 likewise produced separate spores. It is therefore impossible to classify the progeny by tetrads. The gametophytic offspring of this mating were:

Females: Nontufted, 6; tufted, 12; character undetermined, 1.

Males: Nontufted, 6; tufted, 1; character undetermined, 12.

Total females, 19; total males, 19; sex and vegetative character undetermined, 6; grand total, 44.

There was a large proportion of early deaths of clones in this family, especially among the males; this fact accounts for the number whose character was in whole or in part undetermined.

Mating 24. 20.310 (6 percent tufted) × 20.312 (12 percent tufted)

Fertilized March 22, 1923. Sporophytes dried May 8. Forty-six tetrads from one sporophyte sown September 17. Sporelings transplanted December 22, 1923–January 26, 1924.

Forty-eight tetrads from a second sporophyte sown October 24, 1924. Sporelings transplanted February 14–March 27, 1925.

Both parents in this mating were among the offspring of mating 3 (tufted by nontufted; ALLEN 1924). Both have shown consistently a low proportion of tuftedness, except that on 3 occasions small sub-clones of the male parent consisted only of typical or approximately typical branches.

Five tetrads showed apparently aberrant conditions. These were:

(a) A tetrad giving 2 nontufted females, 3 tufted males. When the sporelings were first transplanted, it was noted that 2 of the male plants were closely associated and possibly originally parts of 1 plant. These 2 males are here considered as a single clone.

(b) A tetrad giving 3 tufted females, respectively 1, 2, and 3 percent tufted. The latter 2 are considered as a single clone.

(c) A tetrad giving 3 tufted females, 1 nontufted male. Of the 3 females,

the 2 most closely similar as to proportion of tuftedness (respectively 38 and 39 percent) are treated as a single clone. The tetrad is thus classed as as having yielded 2 tufted females, 1 nontufted male.

(d) A tetrad giving 3 nontufted females, 1 tufted male. The 3 females are considered as representing 2 clones.

(e) A tetrad giving 3 nontufted females; these are treated as 2 clones.

With the emendations noted, the results of mating 24 are shown in table 2, the offspring of spore tetrads from the 2 sporophytes being classed together.

Mating 25. 20.310 (6 percent tufted) \times 20.122 (2 percent tufted)

Fertilized March 22, 1923. Sporophytes dried May 11. Fifty-four tetrads from 1 sporophyte sown October 16, 1924. Sporelings transplanted January 7–March 23, 1925.

The mother was the same as in mating 24. The father was an offspring of mating 8 (tufted, separable \times nontufted; ALLEN 1924).

A large proportion of clones in this family (19 ♀ ♀, 26 ♂ ♂, 4 of undetermined sex) died before their vegetative characters were satisfactorily determined. The number of tetrads that could be classified as to the charac-

TABLE 2

Results of matings of little-tufted \times little-tufted; distribution of offspring according to spore tetrads.

<i>f₁</i>		M 24	M 25	TOTALS
♀	♂			
2 nt.	2 t.	2	.	2
2 nt.	1 t.	3	.	3
2 nt.	.	6	2	8
1 nt.	1 t.	3	.	3
1 nt.	.	12	5	17
..	1 t.	2	1	3 36
2 t.	2 nt.	3	1	4
2 t.	1 nt.	3	.	3
2 t.	.	6	1	7
1 t.	1 nt.	2	.	2
1 t.	..	12	4	16
..	1 nt.	3	..	3 35
Totals		57	14	71

Totals (matings 23, 24, 25): ♀ nontufted clones, 52; ♀ tufted, 58; ♂ nontufted, 22; ♂ tufted, 14. Character not determined: ♀, 21; ♂, 50; sex undetermined, 10. Totals, all offspring: ♀, 131; ♂, 86; sex unknown, 10; grand total, 227.

ter of their offspring is therefore, as appears in table 2, comparatively small.

One clone gave 3 males of undetermined character; they are treated as 2 clones. Another tetrad gave 3 females and 1 male, all of undetermined character; they are treated as 2 females, 1 male.

In families 24 and 25, as shown in table 2 (mating 23 being unanalyzable in terms of tetrads), the character of the offspring is exactly what would be expected from a mating between a tufted and a nontufted parent, on the assumption that the parental difference was due to a single factor. The 2 groups of tetrads expected in such a case—namely, those which *may* be composed respectively of 2 nontufted females, 2 tufted males, and of 2 tufted females, 2 nontufted males—are substantially equal. Table 2, showing the offspring of 2 tufted parents, is thus exactly comparable with the upper 2 divisions of table 1, showing the offspring of matings of nontufted with tufted parents, but lacks entirely the really or apparently aberrant types of intra-tetrad distribution shown in the lower part of table 1. In other words, the results of the mating of 2 tufted parents agree more closely with the expectation, on a simple Mendelian basis, as to the results of the mating of 1 tufted and 1 nontufted parent than do the results of the latter type of mating itself. This unexpected condition might be explained by the assumption that in the matings now in question 1 of the parents had been erroneously classified, having been really nontufted; but the history of both parents in each case negatives such a possibility. Another conceivable explanation would be that the offspring classed as nontufted were really tufted. As previously pointed out, an error of this nature is bound to occur at times in the determination of the character of genetically slightly tufted clones; but the present results are too uniform, and the history of many of the offspring clones has been followed too long, to leave any plausibility in such an assumption. So far as possible, the apparently nontufted offspring were kept in culture much longer than most of those that were obviously tufted, to increase the chance that they might produce tufted branches. A few of the nontufted female clones, indeed, are still living.

Little-tufted ♀ × *Much-tufted* ♂

Mating 26. 21.14 (1 percent tufted) × 20.384 (82 percent tufted)

Fertilized May 12, 1922. Sporophytes dried July 7. Twenty tetrads from 1 sporophyte sown separately December 2. Sporelings transplanted March 29–May 18, 1923. The distribution of the offspring is shown in table 3.

Tetrads from a second sporophyte sown broadcast December 2, 1922. Sporelings transplanted April 5 and 13, 1923. Clones arising from these sporelings were classed as follows:

Females: Nontufted, 12; tufted, 10; character undetermined, 3. Males: nontufted, 8; tufted, 1; character undetermined, 8.

Tetrads from a third sporophyte sown broadcast December 2, 1922. On April 13, 1923, numerous sporelings arising from this sowing were discarded. They included apparently nontufted males, and tufted and nontufted females.

Both parents in this mating were among the offspring of mating 14 (tufted \times polycladous; ALLEN 1925). The mother on July 1, 1921 showed two branches that were classed as tufted. Several later examinations failed to disclose any tufted branches, and it was concluded that the earlier observation had been erroneous. For this reason the clone was treated as "typical" in a previous paper (ALLEN 1924a). On July 27, 1927, tufted branches were again present. The clone must therefore now be considered as tufted.

TABLE 3

Results of mating of little-tufted \times much-tufted; distribution of offspring according to spore tetrads.

<i>f₁</i>		M. 26	
♀	♂		
2 nt.	2 t.	1	
2 nt.	...	3	
1 nt.	2	6
2 t.	1 nt.	1	
2 t.	5	
1 t.	1 nt.	1	
1 t.	1	8
1 nt., 1 t.	1 nt.	1	1
Total		15	

Totals (offspring from spores of 1 sporophyte, shown above, plus spores of a second sporophyte sown broadcast): ♀ nontufted, 23; ♀ tufted, 25; ♂ nontufted, 11; ♂ tufted, 3. Character not determined: ♀, 4; ♂, 15. Totals, all sporelings: ♀, 52; ♂, 29; grand total, 81.

One tetrad of the present family, as noted previously, apparently gave rise to 3 female clones, all tufted. Estimates of the proportions of tuftedness displayed by these clones gave respectively 43, 57, and 59 percent. The two latter, being so similar phenotypically, are assumed to have come from a single plant, and the tetrad is listed as having produced 2 tufted clones.

It will be seen from table 3 that the results of this mating of little-tufted \times much-tufted, so far as can be judged from the relatively small numbers, do not differ materially in character from those shown in previous tables for matings of nontufted \times much-tufted or of little-tufted \times little-tufted.

The 1 "exceptional" tetrad shown in table 3 illustrates the difficulties of classification of clones that are phenotypically near the border line between tuftedness and nontuftedness. One female clone (23.245) from this tetrad was kept in culture for nearly 3 years. On one occasion it showed a few undoubtedly tufted branches; at all other times when it was examined it appeared nontufted. It is classed as 1 percent tufted. The second female clone from the same tetrad (23.246) is still living, being now more than 6 years of age. It has never shown a tufted branch, although on 1 occasion a few "doubtful" branches (showing some marked irregularities), and on another occasion 1 doubtful branch, were observed. It is classed as nontufted. The 1 male clone of this group (23.247) died after but 2 observations of its character had been made; on both occasions it displayed no tufted branches, and it is therefore classed as nontufted. Obviously its determination is not entirely satisfactory.

Much-tufted ♀ ♀ \times *Much-tufted* ♂ ♂

Mating 35. 20.331 (82 percent tufted) \times 20.320 (64 percent tufted)

Fertilized February 24, 1923. Sporophytes dried May 5. Thirty-six tetrads from one sporophyte sown September 14. Sporelings transplanted December 21, 1923–January 26, 1924.

Both parents were among the offspring of mating 3 (tufted \times nontufted; ALLEN 1924). Both produced throughout their lives large proportions of tufted branches.

The offspring of this mating were transplanted when somewhat advanced in growth. In consequence, it appeared from the characters of the respective clones that in a number of instances a plant had been divided before being transplanted. In 3 instances, 3 female clones appeared from a single tetrad instead of the expected 2; in one case there were 4 female clones instead of 2; and in 3 instances 3 male clones appeared instead of 2. Corrections were made in these cases, as previously described. The occurrence of so many errors in isolation of clones makes it not unlikely that now and then the 2 female or the 2 male clones referred to a tetrad may represent really, but 1 clone. In this family, however, the results are so uniform that such errors can not affect the conclusions, except as they might somewhat modify the calculated average percentages of tuftedness (table 5).

The results of this mating are given in table 4.

Mating 36. 20.331 (82 percent tufted) \times 20.111 (74 percent tufted)

Fertilized February 24, 1923. Sporophytes dried May 3. Twenty tetrads from 1 sporophyte sown September 14. Sporelings transplanted December 5, 1923–January 25, 1924.

The mother was the same as in mating 35. The father was an offspring of mating 8 (tufted, separable \times nontufted; ALLEN 1924).

The results are shown in table 4.

Mating 37. 20.313 (55 percent tufted) \times 20.107 (93 percent tufted)

Fertilized February 24, 1923. Sporophytes dried May 8. Forty-five tetrads from 1 sporophyte sown October 17, 1924. Sporelings transplanted February 13–March 24, 1925.

The mother in this mating, like that in mating 35, was an offspring of mating 3. The father, as in mating 36, was an offspring of mating 8.

Germinations in this case, as table 4 shows, were relatively few, and most of the sporelings died while young.

Mating 38. 20.173 (77 percent tufted) \times 20.252 (71 percent tufted)

Fertilized March 8, 1923. Sporophytes dried May 16. Forty-eight tetrads from 1 sporophyte sown October 26, 1924. Sporelings transplanted March 17–28, 1925.

Both parents were among the offspring of mating 14 (tufted \times polycladous; ALLEN 1925).

Only a few germinations were obtained. Of the 2 male clones listed in table 4, 4 observations of 1 clone, and 3 of the other, showed them nontufted.

Mating 39. 20.173 (77 percent tufted) \times 20.384 (82 percent tufted)

Fertilized March 8, 1923. Sporophytes dried May 16. Forty-eight tetrads from 1 sporophyte sown October 26, 1924. Sporelings transplanted February 14–March 28, 1925.

Both parents in this mating were among the offspring of mating 14 (tufted \times polycladous). The mother was the same as in mating 38; the father the same as in mating 26.

One tetrad resulting from mating 39 yielded apparently 3 male and 1 female clones. Of the males, 2 died before their character was determined; the third, on 4 observations, appeared nontufted. These are treated as 2 males, 1 nontufted and 1 of undetermined character. The female clone from this tetrad was polycladous. The possibility of contamination seems to be excluded, since at its first transplanting from the pot in which the

tetrad was sown this female plant was noted as "apparently polycladous"—a determination confirmed by 2 later observations when the clone was large enough to make the diagnosis certain. It must therefore be considered a mutant; possibly the fact may be significant that the mutation was a return to the character of one of its gametophytic great-great-grand-parents. The results, including the 3 tetrad sibs in question, are shown in table 4.

TABLE 4
Results of matings of much-tufted × much-tufted.

<i>f</i> ₁ (BY TETRADES)	NUMBER OF TETRADES					TOTALS
	M.35	M.36	M.37	M.38	M.39	
2 ♀, 2 ♂	5	1	6
2 ♀, 1 ♂	6	6	.	.	3	15
2 ♀ .	3	1	.	..	1	5
1 ♀, 2 ♂	6	2	.	1	1	10
1 ♀, 2 ♂, 1 ?	1	1
1 ♀, 1 ♂	2	3	1	.	.	6
1 ♀ ...	3	4	4	.	8	19
... 2 ♂	1	1	2
... 1 ♂	1	1	1	.	1	4
... 1 ♂, 1 ?	.	..	1	.	.	1
... . 3 ?	1	.	.	1
... .. 1 ?	.	.	6	5	1	12
Totals	28	18	14	6	16	82

Character of f₁ Offspring.

	M.35	M.36	M.37	M.38	M.39	TOTALS
♀ tufted	40	25	2	1	16	84
♀ nontufted	1*	1
♀ undetermined character	..	.	3	.	.	3
♂ tufted	29	9	.	..	4	42
♂ nontufted	2	1	3
♂ undetermined character	6	7	3	..	3	19
Sex and character undetermined	1	..	10	5	1	17
Totals	76	41	18	8	26	169

* Polycladous mutant.

Table 4 shows that the results of matings of much-tufted \times much-tufted are materially different from those of matings of the types previously considered. Of the f_1 female clones whose character was determined with some confidence, only 1 of 85 was classed as nontufted; and that 1 was a mutant in which tuftedness may have been masked by polyclady. Of f_1 males similarly determined, only 3 in 45 were classed as nontufted. The possible failure of a slightly tufted clone to produce tufted branches over a considerable period renders it not unlikely that any or all of these 3 clones may have been genetically tufted. Apart, therefore, from rare exceptions which may involve mutation, matings between much-tufted clones (of the character of those used in the present work) give entirely tufted offspring. This result would naturally be expected of a mating between any 2 tufted clones, at least on the assumption that they bear a common factor for tuftedness; but it is, in fact, very different from the consequences that followed matings of little-tufted \times little-tufted (table 2) or of little-tufted \times much-tufted (table 3). It follows that clones displaying different degrees of tuftedness differ in their genetic make-up, as evidenced by the character of their offspring.

Genotypic differences between parents of different degrees of tuftedness are indicated also by the percentages of tuftedness manifested by their offspring, as shown in table 5 for each of the families thus far considered.

In considering this table, comparison may first be made of the range and percentage of tuftedness characterizing the tufted offspring alone of each group of families. Inspection of the table shows notable differences in these respects, particularly between the offspring of much-tufted \times much-tufted and the offspring of matings of other types. Further light is thrown on these differences by the average tuftedness of the offspring in each group of families. The averages are as follows, computed for the tufted offspring only:

For females: offspring of nontufted \times much-tufted, 11 percent; little-tufted \times little-tufted, 15 percent; little-tufted \times much-tufted, 35 percent; much-tufted \times much-tufted, 45 percent.

For males: offspring of nontufted \times much-tufted, 30 percent; little-tufted \times little-tufted, 45 percent; little-tufted \times much-tufted (3 clones only), 35 percent; much-tufted \times much-tufted, 49 percent.

For all tufted offspring: nontufted \times much-tufted, 15 percent; little-tufted \times little-tufted, 21 percent; little-tufted \times much-tufted, 35 percent; much-tufted \times much-tufted, 47 percent.

The percentages given for female clones are more dependable than those given for males; partly because the number of females is larger, partly be-

TABLE 5
Percentages of tuftedness in offspring of matings involving tuftedness.

	NUMBER TUFTED CLONES	RANGE OF TUFTED- NESS (PERCENT)	AVERAGE (PERCENT)	NUMBER NOTTUFTED CLONES	TOTAL NUMBER (CHARACTERS DETERMINED)	AVERAGE ALL CLASSIFIED CLONES (PERCENT)
Nontufted × Much-tufted						
M. 19	♀	36	Sl.-63	14	62	8
	♂	8	5-43	26	32	6
M. 20	♀	1	1	10	Sl.
	♂	1	41	5	8
M. 21	♀	20	Sl.-46	9	37	5
	♂	8	1-98	39	15	21
M. 22	♀	7	Sl.-10	3	21	1
	♂	2	4-9	6	8	1
Little-tufted × Little-tufted						
M. 23	♀	12	Sl.-53	23	18	15
	♂	1	38	7	5
M. 24	♀	38	Sl.-39	11	75	6
	♂	12	15-90	44	26	20
M. 25	♀	8	2-50	19	17	9
	♂	1	66	3	22
Little-tufted × Much-tufted						
M. 26	♀	25	1-95	35	48	18
	♂	3	6-48	35	14	8
Much-tufted × Much-tufted						
M. 35	♀	40	12-96	57	40	57
	♂	29	9-74	41	29	41
M. 36	♀	25	Sl -69	27	25	27
	♂	9	34-86	65	9	65
M. 37	♀	2	20-75	48	2	48
	♂	0	0	..
M. 38	♀	1	17	1	17
	♂	0	2	0
M. 39	♀	16	15-77	47	17	44
	♂	4	58-87	71	5	57

* Polycladous mutant.

cause a small proportion of tuftedness is less likely to be noted in a male clone.

The figures, making all allowance for the unsatisfactoriness of the absolute values given, render it abundantly evident that there are genetic differences between clones which differ phenotypically in their proportion of tuftedness. The argument would obviously be strengthened if comparison were made between the percentages of tuftedness averaged for *all* offspring, tufted and nontufted, in the respective groups (see the last column of table 5). Such a comparison is, of course, valid only as between the last 3 groups of families, both parents in each of which were tufted.

Likewise suggestive of genetic complexity are the differences in proportion of tuftedness between members of the same family, shown in the column of table 5 headed "range of tuftedness." These differences are emphasized by a comparison of the records of individual clones within a family; while some show marked discrepancies between the results of observations at different times, many clones have displayed consistently over a term of years a uniform tendency toward, respectively, a high, medium, or low degree of tuftedness.

While the averages show a general correspondence in degree of tuftedness between parents and offspring, despite great differences between individual offspring, the correspondence may be less close when comparison is made within single families. Thus, in families 23 and 25, the tufted female offspring were on the average more tufted than either parent, and the single male clone in each family was similarly highly tufted. In these families, as well as in family 24, the maximum tuftedness among either female or male offspring was several times the tuftedness of either parent. Even when the average is calculated to include nontufted as well as tufted offspring (last column, table 5), the degree of tuftedness is higher than that of either parent in the females of family 23, in the males of family 24, and in both females and males of family 25. Among the offspring of matings 35, 36, and 39, in which both parents were much-tufted, the maximum proportion among the offspring of one or the other sex exceeds the proportion of either parent, although the differences here concerned may fall within the limits of possible error in the determination of degrees of tuftedness.

It has been suggested (ALLEN 1924, pp. 565, 566) that a factorial explanation for tuftedness requires the assumption that more than 1 factor is or may be concerned in the production of this character. The original basis for the suggestion was the observation that different clones persistently manifest different degrees of tuftedness. Clones selected as vary-

ing markedly in degree of tuftedness have now been shown to differ in their genetic behavior.

The occasional production from a mating between a nontufted female and a much-tufted male of a tetrad giving rise to 3 or 4 tufted gametophytes (table 1) indicates the presence in the male parent of more than 1 factor determining tuftedness. The results of matings of much-tufted \times much-tufted (table 4) point likewise to the action of 2 or more distinct and cumulative factors, present in this instance in each parent. That the factors borne respectively by the 2 parents in each such mating are not identical is indicated by the differences among the offspring with respect to degree of tuftedness. Were the parents genetically alike, a greater uniformity among the offspring would be expected. If the factors borne by the mother were entirely different from those borne by the father, some proportion of the offspring would be expected to be nontufted. It is true that a very few of the offspring (in 2 families) were so classified, but reasons have been given for suspecting that their classification was erroneous. Hence it is plausible to assume that in each of these matings the parents had 1 or more factors for tuftedness in common, each possessing in addition 1 or more factors that were lacking in the other parent.

However, the genetic character of a majority of the tetrads resulting from a mating of nontufted \times much-tufted (first 2 divisions of table 1) could be explained by the assumption of a *single* differentiating factor. If 2 or more such factors are present in each male parent, a close linkage between these factors must be postulated in order to explain the occurrence of so large a proportion of tetrads each yielding 2 nontufted and 2 tufted clones.

An alternative line of explanation, and one which better fits the results of certain other matings, would assume the presence of at least 1 factor of another type. In the matings summarized in table 1, it may be supposed that each much-tufted father bears 2 or more factors for tuftedness (T_1, T_2, \dots) which may or may not be linked, and in addition a factor R which does not tend in itself to produce tuftedness but is necessary to the appearance of that character. It must also be assumed that R is closely linked with one of the factors of the former group (say T_1). In such a case the majority of the tetrads would give rise each to two nontufted and two tufted clones; and crossing over between T_1 and R would account for an occasional tetrad producing nontufted clones only. The apparently rare production of 3 or 4 tufted clones from a single tetrad might depend upon an additional factor R' , present in certain much-tufted males and closely

linked with T_1 and R , or upon an occasional mutation of r , derived from the nontufted mother, to R .

The results of matings of much-tufted \times much-tufted (table 4) offer no difficulty to conceptions of the nature of those just developed. A crossing over between R and T_1 in the one parent, or between similar factors in the other parent, might result in the appearance of an occasional nontufted clone; otherwise all the f_1 clones would be tufted.

To account on a similar basis for the consequences of matings of little-tufted \times little-tufted (table 2), or of little-tufted \times much-tufted (table 3), further assumptions are required. In the matings of the former type it may be supposed, as before, that 1 parent possesses the factor-complex RT_1 , R being closely linked with T_1 and with other similar factors, if present, but that the other parent has the complex $R'T_n$, R' not being linked with T_n or with any other factor affecting tuftedness. An F_1 sporophyte, then, would have the formula $RrR'r'T_1t_1T_nt_n$. (Capital and lower case symbols here, of course, do not imply dominance and recessiveness, since characters of the haploid generation are in question.) Leaving out of consideration the consequences of a rare crossing over between R and T , as well as the possible occasional occurrence of a four-type tetrad, tetrads of the following constitutions would be formed in equal numbers: $2RT_1R'T_n$, $2rt_1r't_n$; $2RT_1r'T_n$, $2rt_1R't_n$; $2RT_1R't_n$, $2rt_1r'T_n$; $2RT_1r't_n$, $2rt_1R'T_n$. The first, second, and third of these combinations would give rise to 2 tufted clones of 1 sex, 2 nontufted of the opposite sex; the fourth combination, to 4 tufted clones, 2 of either sex. Three-fourths of the tetrads, therefore, would belong to the 2 classes appearing in table 2; one-fourth to a class not there shown to occur. It may be argued that, since the number of tetrads shown in the table is comparatively small, and since in so large a proportion of cases only 1 or 2 spores of a tetrad germinated, giving rise to 1 or 2 tufted clones, it is not impossible that some fraction of the tetrads resulting from these matings were actually genetically all tufted. This possibility, that of the occurrence of crossing over, and that of the appearance of four-type tetrads, require testing by means of much larger families.

The hypothesis could be further developed by assuming additional factors necessary, in addition to R' , for the appearance of tuftedness; with appropriate assumptions as to linkage, the expected proportions of tetrads producing all tufted clones could thus be reduced indefinitely.

An explanation thus developed, while perhaps formally satisfactory, requires many assumptions that are as yet unsupported. On the other hand, certain results of matings involving the "semisterile" and "cupulate"

characters have suggested the occurrence in *Sphaerocarpos* of a method of inheritance which may possibly play a rôle in the phenomena here under discussion.

Both semisterility and cupulateness have appeared in male gametophytes only. The former, distinguishing several clones that probably had a common origin, was described by WOLFSON (1925). The latter character has appeared once in a mutant (from mating 31, described later in the present paper). Both characters are constant and are inherited indefinitely through vegetative multiplication; semisterile clones have been in continuous culture since 1921; the cupulate clone, since 1924. None of the offspring of matings of semisterile or of cupulate males have displayed the paternal character. For example, a mating of a semisterile male with a tufted female gives rise to tufted and nontufted gametophytes of either sex, but to none that show the semisterile character. The possible explanation that spores carrying the semisterile tendency are nonviable is negated by the fact that in some instances germination has been secured of 3 or of 4 spores from a single tetrad. Equally conclusive evidence is not yet available from matings involving the cupulate character.

More or less closely analogous occurrences of matrocliny in angiosperms have been explained by cytoplasmic influence; either the material basis of inheritance (for example, of plastid characters) is thought to reside in the cytoplasm of the egg, or the maternal cytoplasm is considered to exert an inhibiting influence upon the expression of factors contributed by the male gamete. It is possible that an explanation of this general nature may apply to the unexpected results of matings involving semisterility and cupulateness. Or, quite differently, the X-chromosome contributed by the egg may be found to play some preponderant rôle in inheritance proportional to its bulk.

Returning to the results of matings between little-tufted and little-tufted (table 2), and of that between little-tufted and much-tufted (table 3), it is clear that their explanation would be much simplified if it could be supposed that a factor or a factor-complex received from 1 parent may be inactivated or its expression inhibited in consequence of gametic union. This supposition might leave the results of all the matings involving tuftedness explainable in terms of multiple factors determinative for various intensities of that character. The supposed inactivation of factors coming from 1 parent could not, however, apply to all the factors furnished by that parent, nor even to all the factors affecting tuftedness; for, in matings involving a high degree of tuftedness on both sides (table 4), if all such factors in either parent were to become functionless, some con-

siderable proportion of the offspring would be expected to be nontufted; and this is clearly not the case. Likewise, in certain matings involving polyclady reported later in the present paper, in which 1 parent was genetically tufted, there is no evidence of a suppression in the offspring of the tufted tendency, from whichever parent that tendency was inherited.

THE POLYCLADOUS CHARACTER

Polycladous plants, as already described (WOLFSON 1925, ALLEN 1925), differ from typical gametophytes in frequency of lobing and branching; in the forms of lobes and of involucre; and in the presence of numerous dorsal lobes and cilia. The males bear fewer antheridia than typical males, considerable portions of a culture often lacking antheridia entirely. The females bear only extremely rare archegonia and in numerous attempts at mating have proved entirely sterile. Polyclady, differently from tuftedness, is constant in expression. A polycladous clone, male or female, produces only polycladous branches, although rarely an involucre appears of approximately typical form. All my polycladous plants are descended from two male clones which probably had a common origin.

Previous work has shown that polyclady behaves in matings as though the difference between it and the typical condition depended upon a single factor. With this conclusion the present results are in agreement.

The apparent simplicity of the method of inheritance of this character suits it particularly for the study of the intra-tetrad distribution of the genetic factors involved. In table 6 all the material available to date for such an analysis is brought together. The figures repeated in this table from previous papers are those from matings 12-15 (ALLEN 1925) and 28-32 (ALLEN 1926a). Two corrections have been made in the report of mating 32, as noted below in the discussion of that mating.

From matings involving both tuftedness and polyclady (ALLEN 1925), the expected class of offspring showing both these characters could not be recognized. It was concluded provisionally that "the aberrancies due to the inheritance of tuftedness are masked by the greater and uniformly present aberrancies of polyclady." This conclusion is confirmed by the results of matings 27 and 31-34 (below); the three polycladous males shown by these results to be also genetically tufted were among the progeny of mating 14 (nonpolycladous, tufted \times polycladous). In table 6, only the results bearing upon the inheritance of polyclady (or its absence) are included.

Mating 40 involves also the character known as "appendiculate." The distinction between appendiculate and non-appendiculate clones is difficult

and requires the study of more, and more prolific, matings than have yet been obtained. Its discussion is therefore deferred to a later report.

In the cases of 3 sowings [from matings 27, 32, and 34, all nonpolycladous, nontufted \times polycladous (tufted)] made on October 21, 22, and 23, 1923, it was evident from the numbers of sporelings appearing that 2 or more sets of tetrads had been sown in some or all of the pots. Since uncertainty was thus introduced as to intra-tetrad distribution, the results of these sowings, though summarized in table 8, are omitted from the classification in table 6.

Nonpolycladous ♀ ♀ \times *Polycladous* ♂ ♂

Mating 12. 19.24 \times R19 δ 1aB

Mating 13. 19.6 \times R19 δ 1aA

These matings were reported earlier (ALLEN 1925).

The tetrads from 1 sporophyte of mating 12, sown broadcast, gave only 1 sporeling, a nonpolycladous male.

Spore tetrads from 3 sporophytes resulting from mating 13 were sown separately. One spore from each of 4 tetrads germinated, 3 spores giving rise to nonpolycladous females, 1 to a polycladous male. Tetrads from 5 sporophytes, sown broadcast, gave more extensive results (table 1, ALLEN 1925). Since the tetrad relationships of the offspring of these spores could not be determined, they are not classified in table 6 but are summarized in table 8.

Mating 28. 20.337 \times 21.148

Fertilized March 31, 1922. Sporophytes dried July 7. Twenty tetrads from 1 sporophyte sown November 8. Sporelings transplanted April 13–May 17, 1923.

Both parents were among the offspring of mating 13 (above). The mother in the present mating was one of the clones which, because of the presence at times of an unusual number of aberrant involucre, was classed provisionally as “possibly atypical.” But the offspring of a mating of this clone with a typical male (mating 16, ALLEN 1926) showed it to be genetically indistinguishable from a typical clone.

Mating 29. 21.85 \times 20.266

Fertilized May 12, 1922. Sporophytes dried July 7. Twenty tetrads from 1 sporophyte sown November 7. Sporelings transplanted April 12–May 3, 1923.

The mother was an offspring of a mating of typical parents (mating 1; ALLEN 1924). The father was an offspring of mating 13, noted above.

Mating 30. 21.153×20.266

Fertilized May 12, 1922. Sporophytes dried July 12. Twenty spore tetrads from 1 sporophyte sown January 12, 1923. Sporelings isolated May 18.

The mother in this mating was among the offspring of mating 13. It was one of those first classed as "possibly atypical," but its breeding behavior (mating 17; ALLEN 1926) showed it to be genetically indistinguishable from typical clones.

The father was the same as in mating 29.

Nonpolycladous, Appendiculate ♀ × *Polycladous* ♂

Mating 40. PSC2Bδ1.8b×20.266

Fertilized February 10, 1926. Sporophytes dried May 21. Thirty-five tetrads from 1 sporophyte sown separately November 8. Sporelings isolated May 24–28, 1927.

The mother in this mating was an offspring of fertilization within a culture of male and female plants received from Miami, Florida, in 1916. This clone has been preserved because it has shown rather consistently an unusually large proportion of involucre appendages, as well as other minor irregularities in the form of its involucre. In the present paper, the question of the inheritance of the appendiculate character will not be considered.

The father in this mating was the same as in matings 29 and 30.

The classification of 1 male offspring (clone 27.210) is still somewhat uncertain. It resembles polycladous males in most respects, but produces an unusually large proportion of involucre approaching more or less closely the typical form. It has given sporophytic progeny in 1 mating with a typical female and a few spores have germinated. It is impossible to determine from the nature of the still young plants arising from these spores the genetic constitution of their male parent. This clone is classed in table 6 as polycladous. Since it was 1 of only 2 male progeny of mating 40, the other being nonpolycladous, the possibility is open that its character is a resultant of the presence of appendiculate and polycladous factors.

Nonpolycladous, Tufted ♀ × *Polycladous, Nontufted* ♂ ♂

Mating 14. R35G1 (67 percent tufted) × R19δ1aA

Mating 15. R27E (53 percent tufted) × R19δ1aB

The details of these matings and the source of the parents were reported earlier (ALLEN 1925).

Thirty-two and 16 tetrads, derived respectively from 2 sporophytic

offspring of mating 14, were sown separately. The offspring arising from these spores are classified in table 6. Tetrads from a third sporophyte were sown broadcast. The results of their germination are included in table 8.

Since the mother in mating 15 carries the spore-separation tendency, the spores borne by its sporophytic progeny were not united in tetrads. Spores from 3 of these sporophytes were sown broadcast. Among the offspring of spores from 1 sporophyte were tufted and nontufted but no polycladous clones. Since a doubt is thus suggested as to the paternity of this sporophyte, its gametophytic offspring are not here recorded; those of the other 2 sporophytes are summarized in table 8.

Nonpolycladous, Nontufted ♀ ♀ × *Polycladous (Tufted)* ♂ ♂

The mothers in this series of matings were all strictly typical. The fathers were among the progeny of mating 14 (above). Although they were not distinguishable phenotypically from other polycladous males, their nonpolycladous offspring include both nontufted and tufted clones. It is evident, therefore, that the polycladous fathers in the present matings were genetically tufted.

Mating 27. 21.45 × 21.13

Fertilized March 8, 1923. Sporophytes dried May 8. Forty-eight tetrads from 1 sporophyte sown October 23. Sporelings isolated January 24–March 27, 1924.

The mother was an offspring of mating 13; the father, of mating 14.

This is one of the families in which the numbers of sporelings appearing made it evident that in several instances 2 or more tetrads had been sown in a single pot. This family is therefore not included in table 6 but is summarized in table 8.

Mating 31. 20.205 × 20.254

Fertilized March 8, 1923. Sporophytes dried May 9. Sixty tetrads from 1 sporophyte sown September 27. Sporelings isolated January 17–February 5, 1924.

Both parental clones were among the progeny of mating 14.

One spore tetrad in this family apparently produced 3 nonpolycladous females; they are classed as 2 clones. One apparently produced 4 nonpolycladous males; they are classed as 2.

One male offspring (23.7094), classed in table 6 as nonpolycladous, is a new mutant form. The antheridia in this clone are about as abundant as in a typical clone, but are relatively small. The antheridial involucre,

while variable in form, are prevailingly expanded into cup- or saucer-like structures, at the center of each of which a partly exposed antheridium is attached. This mutant clone, referred to as "cupulate," has been mated with 6 different females. Sporophytes resulted from 2 of these matings; the nature of their gametophytic offspring has been noted on a previous page.

Mating 32. 20.205 \times 20.255

Fertilized March 8, 1923. Sporophytes dried May 21. Forty-eight tetrads from 1 sporophyte sown October 21. Sporelings isolated January 22–February 5, 1924.

Twenty-four tetrads from a second sporophyte sown October 24, 1924. Sporelings isolated February 14–March 27, 1925.

Both parents were among the progeny of mating 14. The mother was the same as in mating 31.

Of the offspring of the spores sown in 1924, 7 appeared in one pot and 6 in another, although only 1 tetrad was supposed to have been sown in each. Since it was evident that each of these two pots at least had received more than 1 tetrad, all the results of the sowings of 1924 are omitted from table 6, but are included in table 8.

Among the offspring of the sowing of 1923 the following apparent discrepancies occurred. One tetrad yielded 2 female nonpolycladous, 1 female polycladous, 1 male polycladous; the 2 nonpolycladous females are considered as 1 clone. One tetrad yielded 3 nonpolycladous females, classed as 2 clones. One tetrad yielded 3 nonpolycladous females (classed as 2) and 1 polycladous male.

The classification of the offspring of this mating (sowing of 1923) as shown in table 6 differs from that given in a previous paper (ALLEN 1926a, table 1) in two respects. One tetrad previously classed as yielding 1 polycladous female, 1 nonpolycladous male is now credited with 1 nonpolycladous male only, because of the unsatisfactory determination of the (probable) female. One tetrad, yielding 1 nonpolycladous female, 1 polycladous female, 1 nonpolycladous male is included which was omitted from the previous tabulation.

Mating 33. 21.45 \times 20.254

Fertilized March 8, 1923. Sporophytes dried May 11. Forty-eight tetrads from 1 sporophyte sown October 2, 1924. Sporelings isolated February 21–March 26, 1925.

The mother in this mating was the same as in mating 27. The father was the same as in mating 31.

One tetrad in this family yielded apparently 3 polycladous males (classed as 2) and 1 nonpolycladous female.

Mating 34. 21.45×20.255

Fertilized March 8, 1923. Sporophytes dried May 8. Forty-eight tetrads from 1 sporophyte sown October 22, 1924. Sporelings isolated January 7–March 26, 1925.

The mother was the same as in matings 27 and 33; the father, the same as in mating 32.

This family represents another instance in which more than 1 set of tetrads were evidently sown in the same pots. For this reason the results are not included in table 6, but are summarized in table 8.

TABLE 6

Results of matings of nonpolycladous \times polycladous; distribution of offspring according to spore tetrads. (M. = mating; np. = nonpolycladous; p. = polycladous).

f_1		M. 12, 13	M. 14	M. 28	M. 20	M. 30	M. 31	M. 32	M. 33	M. 40	TOTALS
♀	♂										
2 np.	2 p.	4	2	..	.	6
2 np.	1 p.	3	4	.	.	7
2 np.	1	1	.	3	3	4	.	12
1 np.	2 p.	1	.	2	1	3	.	7
1 np.	1 p.	..	.	1	..	.	2	2	2	..	7
1 np.	3	7	2	2	..	4	3	7	6	34
....	2 p.	..	.	1	3	.	..	3	.	.	7
....	1 p.	1	2	2	.	3	..	8 88
2 p.	2 np.	2	2
2 p.	1 np.	3	.	.	3
2 p.	1	1	.	1	3
1 p.	2 np.	1	.	1	1	..	.	3
1 p.	1 np.	1	3	..	4*	2	..	.	10
1 p.	1	4	1	.	1	4	2	3	16
..	2 np.	.	..	1	1	.	4	1	1	.	8
....	1 np.	1	..	2	2	.	2	2	4	1	14 59
1 np., 1 p.	1 np., 1 p.	1	.	.	.	1	.	..	2
1 np., 1 p.	1 p.	1	.	..	1
1 np., 1 p.	1	1
1 np.	1 np., 1 p.	1	..	1	.	2
1 np.	1 np.	1	..	1	..	2
1 p.	1 np., 1 p.	2	.	..	2
1 p.	1 p.	1	..	1†	2 12
Totals		5	9	14	15	2	37	37	28	12	159

* One "nonpolycladous" ♂ is a cupulate mutant.

† Polycladous ♂ of somewhat uncertain character.

One important difference is to be noted between the inheritance of polyclady and that of tuftedness. From matings of nontufted \times tufted (table 1), the class of tetrads whose spores are genetically 2 nontufted female, 2 tufted male, and that class yielding 2 tufted females, 2 nontufted males seem to be approximately equal. On the other hand, from matings of non-polycladous \times polycladous (table 6), the corresponding classes of tetrads, one giving 2 nonpolycladous females, 2 polycladous males, the other 2 polycladous females, 2 nonpolycladous males, appear to be very unequal. In the latter case the results may be better judged if only those tetrads are considered whose genetic constitution is beyond question—on the assumption, supported by all the evidence now at hand, that of the spores of each tetrad 2 are genetically female and 2 male, 2 nonpolycladous and 2 polycladous. Accepting this assumption, the character of the spores of a tetrad is established if all 4, or only 3, germinated; or if 2 germinated, giving rise either to 2 female, 2 male, 2 nonpolycladous, or 2 polycladous clones. The tetrads represented in table 6, the germination of whose spores met one of these requirements, are of the 3 classes shown in table 7.

TABLE 7

Genetic types of spore tetrads (decisive cases only) among offspring of nonpolycladous \times polycladous.

MATING	2np., σ^2 2p.	♀ 2p., σ^2 2np.	♀ 1np., 1p., σ^2 1np., 1p.	TOTALS
14	.	1	..	1
28	2	1	.	3
29	5	2	1	8
31	12	7	3	22
32	13	6	5	24
33	7	1	2	10
40	..	1	1	2
Totals	39	19	12	70
Percentages	56	27	17	100

The suggestions of table 6 are confirmed by table 7. It is shown that, among the tetrads produced by the sporophytic offspring of a mating of nonpolycladous \times polycladous, those whose (genetically) female spores are nonpolycladous and whose male spores are polycladous are about twice as numerous as those in which the reverse relation holds between vegetative character and sex, and that the third class, whose four spores are all genetically different, are fewer than those of either of the two-type classes, these four-type tetrads constituting about one sixth of the total number. These proportions are not very different from those based upon more limited

numbers as given previously (ALLEN 1926a); at that time the numbers for the corresponding classes were respectively 32, 16, and 8. The chief difference resulting from the inclusion of additional results is that the proportion of four-type tetrads is raised from one seventh to about one sixth of the total.

Of the classes of tetrads in question, the first is that in which the character-pairs, femaleness-maleness and polyclady-nonpolyclady, are represented in each tetrad in the combinations in which they occurred in the gametophytic parents; the second class is that in which the parental characters appear in new combinations. That tetrads of the first class are about twice as numerous as those of the second implies a linkage of some nature between factors for sex and those determining polyclady or non-polyclady, a severing of this linkage occurring during meiosis in about one third of the spore mother cells.

If sexual characters were not involved, the natural assumption would be that the two character-pairs concerned are represented by factors borne on the same pair of chromosomes, the breaking and recombination of their linkages being explainable by the familiar hypothesis of crossing over. But it has been shown (ALLEN 1919; LORBEER 1927) that the influences differential for sex in this species reside in the X- and Y-chromosomes. These chromosomes are so different in size as to make it difficult to imagine an intertwisting and an exchange of corresponding parts between them. It is at least equally possible that the partial linkage here demonstrated is one between different chromosomes, these chromosomes tending to segregate in such a manner in meiosis that the parental grouping (in the case of the X-Y and one other chromosome-pair) persists about twice as often as it is broken. A possible mechanism for such a persistence of parental chromosome groupings is suggested by the end-to-end connections of chromosomes observed in several plants and most fully studied by CLELAND (1923 and later papers) and others in *Oenothera*. That not all the chromosomes of *Sphaerocarpos* tend to retain their parental groupings during meiotic segregation is indicated by the inheritance of tuftedness or nontuftedness independently of sex.

The relation of the production of tetrads of the third (the four-type) class to occurrences during meiosis has already been discussed (ALLEN 1924a, 1926a). Segregation of chromosomes or of chromosome parts limited to the heterotypic division must produce a tetrad composed of spores of only 2 genetically different types. The occurrence of 4 types in a single tetrad implies, therefore, some qualitative segregation in the homoeotypic division. That segregation in the present case occurs ex-

clusively in the homoeotypic division is rendered unlikely, as already pointed out, by the relatively small proportional occurrence of four-type tetrads. It is negatived also by the extensive cytological evidence for the heterotypic segregation of chromosomes in plants, as well as by the demonstrated fact that in *Sphaerocarpus Donnellii* the X- and Y-chromosomes separate in the heterotypic division (ALLEN 1919; LORBEER 1927). It is concluded therefore that some form of segregation may occur in each division. A similar conclusion has been drawn by Miss NEWTON (1926) from her study of factors affecting syngamy in *Coprinus lagopus*. The segregation here in question may imply the separation of some chromosome pairs in the heterotypic, and of some pairs, regularly or occasionally, in the homoeotypic division (as described for some Metazoa; McCLUNG 1914); or a regular segregation of chromosomes in the heterotypic division, preceded by a crossing over in the "four-strand" stage (BRIDGES 1916).

That four-type tetrads result also from matings involving tuftedness has been noted as probable in the discussion of the inheritance of that character.

Adopting the ratios found in table 7 between numbers of tetrads of the 3 possible classes, the proportion of clones of each type to be expected in an f_1 population derived from a mating of nonpolycladous \times polycladous is 9 ♀ nonpolycladous: 5 ♀ polycladous: 5 ♂ nonpolycladous: 9 ♂ polycladous. Incidentally, exactly the same proportion of types will result if the ratio 4:2:1, based upon more limited results (ALLEN 1926a), holds as between the respective classes of tetrads.

Table 8 gives the classification of all the f_1 offspring yielded by matings of this nature, including both those which could and those which could not be classified according to tetrad relationships. Applying the proportions just calculated to the totals in table 8, it appears that the relative numbers among females are substantially as expected, the actual numbers being 259 nonpolycladous, 155 polycladous, whereas a 9:5 ratio would give 266 nonpolycladous, 148 polycladous. The males, however, do not fit the expectation; there are 173 nonpolycladous and 158 polycladous, instead of 118 and 213, as calculated from a 5:9 ratio. With respect to this discrepancy it is to be noted, first, that females are more vigorous than males and more likely to survive to a time when their character may be determined. They should, therefore, present a more accurate picture of the distribution of characters within a given population. Second, the preponderance of nonpolycladous males apparent in table 8 is due to the earlier matings (12-15); if only the later matings were considered, the balance would swing somewhat in favor of polycladous males (nonpoly-

cladous 106, polycladous 117). Third, because of the lesser viability of males, most if not all of the 11 polycladous clones whose sex was undetermined were probably males. Most of the 84 whose sex and vegetative character were both undetermined were also in all probability males; but no conclusion is justified as to whether the greater number of these were nonpolycladous or polycladous. Apparently some portion of the departure from the expected ratios must be explained by assuming that spores bearing the genetic bases for maleness and polyclady germinate in smaller propor-

TABLE 8
Results of matings of nonpolycladous × polycladous; total offspring.

MATING	♀ np.	♀ p.	♂ np.	♂ p.	p. (sex?)	UNDETERMINED	TOTALS
12	.	..	1	1
13	46	28	28	21	3	11	137
14	17	9	9	7	..	11	53
15	34	30	29	13	3	28	137
27	19	14	14	12	..	4	63
28	6	6	6	4	..		22
29	5	5	9	8	..	2	29
30	2	.		2
31	31	11	22*	20	..	3	87
32	39	24	23	25		12	123
33	22	2	8	12	5	9	58
34	34	20	23	33	..	3	113
40	6	6	1	1†	..	1	15
Totals	259	155	173	158	11	84	840
Expected Proportions	9	5	5	9			

* Includes one cupulate mutant.

† Male of somewhat uncertain character.

tions than do those that are genetically male and nonpolycladous; or that polycladous males are less viable in their very early stages. The latter suggestion is somewhat weakened by the observed fact that in general, after they are well started in growth, polycladous are more resistant than nonpolycladous males to unfavorable conditions.

INTERRELATIONS OF TUFTEDNESS AND POLYCLADY

The fact that polyclady masks the appearance of tuftedness interferes with the determination of possible genetic relations between these two characters. However, from their distribution, so far as it may be observed, in tetrads resulting from matings in which each character was contributed by but 1 parent, certain conclusions are possible. For the purpose of such

a study, material is available from family 14 (nonpolycladous, tufted \times polycladous, nontufted) and from families 31–33 [nonpolycladous, nontufted \times polycladous (tufted)]. Those tetrads of these families which yielded at least 2 fully identifiable sporelings each are classified in table 9.

In this table the tetrads are arranged in 3 groups: (1) those certainly or possibly two-type tetrads giving rise to nonpolycladous females and polycladous males (series a-k); (2) certainly or possibly two-type tetrads, producing polycladous females and nonpolycladous males (series l-r); and (3) apparently four-type tetrads (series s- β). In harmony with the more extensive results based upon the distribution of polyclady alone, tetrads of the first group are in this instance twice as numerous as those of the second.

For purposes of further analysis, it may be assumed as before that the spores of each tetrad are, genetically, 2 nonpolycladous and 2 polycladous, 2 female and 2 male; and, in addition, 2 nontufted and 2 tufted. The latter assumption would fit the very large majority of cases, as shown by the results summarized in table 1, but leaves out of consideration the infrequent appearance, as shown in that table, of a tetrad of 3 or 4 genetically tufted spores (or possibly, also, of 3 or 4 nontufted spores). Omitting these rare occurrences, 4 classes of two-type tetrads and 8 classes of four-type tetrads may be expected to result from matings like those here in question.

Of the tetrads listed in table 9, some give definite results with reference to the 3 pairs of characters concerned; others do not. For example, in series a, b, and c of that table it may be concluded (granting the assumptions just stated) that both genetically male spores of each tetrad were polycladous and tufted. As to series d there is no such certainty, since of the 2 polycladous male spores both may have been tufted, or 1 tufted and 1 nontufted, the tuftedness or nontuftedness of 1 female spore not having been determined. Selecting, therefore, only those tetrads which can be classified with some confidence, the following results are obtained. (Abbreviations are those used in the tables.)

Of the two-type tetrads, that class composed of 2 ♀ np.nt. and 2 ♂ p.t. is represented by series a, b, and c in table 9, a total of 6 tetrads.

The class composed of 2 ♀ np.t. and 2 ♂ p.nt. is represented by series f, g, and h, a total of 10.

The class composed of 2 ♀ p.nt. and 2 ♂ np.t. is represented by the 1 tetrad of series n.

The class composed of 2 ♀ p.t. and 2 ♂ np.nt. is represented by series o and r—3 tetrads.

Thus all the predicted two-type classes are present. The numbers are

TABLE 9

Results of matings involving tuftedness and polyclady; distribution of offspring according to spore tetrads.

<i>f₁</i>		M. 14	M. 31	M. 32	M. 33	TOTALS
♀	♂					
(a) 2np.nt.	2p.	..	2	2
(b) 2np.nt.	1p.	..	2	2
(c) 2np.nt.	1	1	..	2
(d) 1np.nt.	2p.	..	2	..	1	3
(e) 1np.nt.	1p.	..	2	2
(f) 2np.t.	2p.	..	2	2	..	4
(g) 2np.t.	1p.	..	1	3	..	4
(h) 2np.t.	2	..	2
(i) 1np.t.	2p.	1	..	1
(j) 1np.t.	1p.	3	..	3
(k)	2p.	..	.	3	2	5 30'
(l) 2p.	1	..	2	.	3
(m) 1p.	1np.t.	1	.	1
(n)	2np.t.	1	.	1
(o) 2p.	2np.nt.	..	1	1
(p) 2p.	1np.nt.	..	1	1	.	2
(q) 1p.	1np.nt.	.	5*	..	.	5
(r)	2np.nt.		2	.	.	2 15
(s) 1np.nt. } 1np.t. }	2	2
(t) 1np.nt. } 1p. }	1	1
(u) 1np.nt.	1np.nt.	..	1	..	.	1
(v) 1np.t. } 1p. }	{ 1np.nt. 1p.	1	..	1
(w) 1np.t. } 1p. }	1p.	1	..	1
(x) 1np.t.	{ 1np.nt. 1p.	..	1	..	.	1
(y) 2p.	{ 1np.nt. 1np.t.	1	..	1
(z) 1p.	{ 1np.nt. 1p.	1	..	1
(α) 1p.	{ 1np.t. 1p.	1	..	1
(β) 1p.	1p.	1	..	1 11
Totals		1	26	26	3	56

* Including one cupulate male mutant.

small, but, as expected, those producing nonpolycladous females and polycladous males are more numerous than those producing polycladous females and nonpolycladous males.

Of the 8 possible four-type classes, at least 4 are represented, assuming the determinations of tufted and non-tufted clones to have been correctly made.

The 2 tetrads of series s belong to the class 1 ♀ np.nt., 1 ♀ np.t., 1 ♂p.nt., 1 ♂p.t.

The single tetrad of series y represents the class 1 ♀ p.nt., 1 ♀ p.t., 1 ♂np.nt., 1 ♂np.t.

The 1 tetrad of series u represents the class 1 ♀ np.nt., 1 ♀ p.t., 1 ♂np.nt., 1 ♂p.t.

The 1 tetrad of series v and the 1 of series x belong each to one or the other of the following classes: 1 ♀ np.t., 1 ♀ p.t., 1 ♂np.nt., 1 ♂p.nt.; or 1 ♀ np.t., 1 ♀ p.nt., 1 ♂np.nt., 1 ♂p.t.

In view of the limited number of definitely classifiable tetrads, it is striking that 8 of the 12 theoretically possible tetrad types are recognizably present, the missing ones being necessarily of infrequent occurrence. It can hardly be questioned that in larger families the remaining classes also would appear.

Repeated but unsuccessful attempts have been made to discover visible criteria for distinguishing as between polycladous clones of either sex those which are genetically tufted and those which are genetically nontufted.

As shown above, on the basis of the observed ratio between various possible classes of tetrads, the expected proportions of the different types of f_1 clones from matings of nonpolycladous \times polycladous would be 9 ♀ nonpolycladous: 5 ♀ polycladous: 5 ♂nonpolycladous: 9 ♂polycladous. If the matings also involve tuftedness, introduced either by the female or by the male parent, tuftedness being inherited independently of either sex or polyclady, then each type of clone listed above would be replaced by nontufted and tufted types equal in number. But since polyclady masks tuftedness, the polycladous nontufted and polycladous tufted females would, phenotypically, constitute a single class, as would likewise polycladous nontufted and polycladous tufted males. The expected proportion, in a sufficiently large f_1 population, then, would be 9 ♀ nonpolycladous nontufted: 9 ♀ nonpolycladous tufted: 10 ♀ polycladous: 5 ♂nonpolycladous nontufted: 5 ♂nonpolycladous tufted: 18 ♂polycladous. Table 10 shows the characters of all f_1 clones that were identifiable with reasonable confidence from families in which tuftedness and polyclady were involved.

The agreement between results and calculation is perhaps closer than might have been expected. Allowance must of course be made for the smaller numbers obtained of males than of females. The widest divergence from expectation is in the inequality between the first and second types of females. A reference to the proper categories of the partly identified clones listed at the foot of the table might bring the proportions of the respective types closer to expectation; it might, to be sure, conceivably work in the opposite direction.

TABLE 10

Results of matings involving polyclady and tuftedness; total fully identified offspring.

MATING	♀ np.nt.	♀ np.t.	♀ p.	♂ np.nt.	♂ np.t.	♂ p.	TOTALS
14	8	8	9	4	4	7	40
15	14	19	30	10	8	13	94
27	7	12	14	4	5	12	54
31	19	12	11	16		20	78
32	5	32	24	7	8	25	101
33	1	..	2		..	12	15
34	5	23	20	4	3	33	88
Totals	59	106	110	45	28	122	470
<i>Expected Proportions</i>	9	9	10	5	5	18	

♀ nonpolycladous (nontufted or tufted?), 31.

♂ nonpolycladous (nontufted or tufted?), 55.

Polycladous (undetermined as to sex, and as to nontuftedness or tuftedness), 8.

SUMMARY

1. Matings of nontufted × much-tufted result in the production of spore tetrads of which the majority yield, in about equal numbers of cases, respectively, 2 ♀ nontufted and 2 ♂ tufted, or 2 ♀ tufted and 2 ♂ nontufted. Of apparently "exceptional" tetrads, a considerable proportion would probably fall into one or the other of the 2 classes mentioned if all the genetically tufted clones could be recognized as such. A small number of tetrads, however, include each 3 or 4 genetically tufted spores; and probably an occasional one includes 3 or 4 genetically nontufted spores. There is evidence of the infrequent occurrence of four-type tetrads—each yielding 1 ♀ nontufted, 1 ♀ tufted, 1 ♂ nontufted, and 1 ♂ tufted.

2. Matings of little-tufted × little-tufted and of little-tufted × much-tufted produce, so far as available evidence shows, equal numbers of tetrads of the first 2 classes above mentioned.

3. Matings of much-tufted × much-tufted yield solely, or almost solely,

tufted offspring. Apparent exceptions were 1 female mutant, and 3 male clones which may really have been genetically tufted.

4. Among the offspring of each mating, individual clones differ greatly in the degree of expression of the tufted character.

5. In general, the average degree of tuftedness in an f_1 family varies in the same direction as the degree of tuftedness of the parents.

6. A hypothesis is developed for the explanation of the inheritance of tuftedness, involving multiple and complementary factors and certain assumptions regarding linkage.

7. This hypothesis may be simplified if it be supposed, as suggested by the results of certain matings involving 2 other characters, that factors derived from 1 parent may be inactivated, or their expression inhibited, by (possibly cytoplasmic) influences derived from the other parent.

8. Matings of non-polycladous \times polycladous, whether or not other character-differences are involved, result in the production of tetrads of 3 classes, yielding respectively: 2 ♀ nonpolycladous, 2 ♂ polycladous; 2 ♀ polycladous, 2 ♂ nonpolycladous; and 1 ♀ nonpolycladous, 1 ♀ polycladous, 1 ♂ nonpolycladous, 1 ♂ polycladous. About twice as many of the first-named class of tetrads are produced as of the second; those of the third class (four-type tetrads) constitute about one sixth of the total number.

9. The proportions of tetrads in classes 1 and 2 indicate a partial linkage between polyclady (or its absence) and sex. Since the occurrence of crossing over between the large X- and the small Y-chromosome seems improbable, it is suggested that linkage in this case may imply a tendency for at least 2 of the chromosomes received from either parent to pass to the same pole in each meiotic division.

10. The occurrence of tetrads of the third class implies that some segregation of chromosomes or of chromosome parts occurs in the homoeotypic division. Reasons are given for concluding that segregation does not take place exclusively in that division; the observed facts are explainable either by the segregation of 1 chromosome pair in the heterotypic, and of another, regularly or occasionally, in the homoeotypic division, or by a crossing over in the four-strand stage.

11. From the relative numerical occurrence respectively of tetrads of the 3 classes, it is calculated that the 4 possible clonal types should appear in a sufficiently large f_1 population in the following proportions: 9 ♀ nonpolycladous: 5 ♀ polycladous: 5 ♂ nonpolycladous: 9 ♂ polycladous. A tabulation of all f_1 progeny of matings involving polyclady shows a close agreement with the calculated ratio on the part of female clones, but a considerable divergence on the part of males. It is pointed out that, for vari-

ous reasons noted, the classification of female is more reliable than that of male clones.

12. In families to which tuftedness and polyclady were contributed, each by 1 parent, if the 2 characters are separately inherited, apart from certain combinations necessarily infrequent, 4 classes of two-type tetrads and 8 classes of four-type tetrads would be expected. A difficulty is interposed by the masking of the tufted character by polyclady; but the available facts indicate that all 4 expected classes of two-type tetrads and at least 4 of the 8 expected classes of four-type tetrads are produced.

13. In the work reported in the present paper, 1273 f_1 clones have been determined as to both sex and vegetative character. Among these were 5 apparent mutants: 2 appendiculate females, 1 polycladous female, 1 dwarf male, and 1 cupulate male. The 2 male mutants represent forms that have never otherwise been observed.

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STUDIES ON THE VIGOR OF SILKWORMS, *BOMBYX MORI* L

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INTRODUCTION

With the aim of investigating the cause of the alternation of voltinism, which has not yet been cleared up, I have already tried the ovarian transplantation between univoltine and bivoltine larvae and have found that the eggs laid from the transplanted ovaries are of the same nature in voltinism as the eggs laid by the host from its ovaries, that is, the phenomenon is conditioned by the nature of the blood of the foster mother, and this made it clear that, as the apparent matrocliny in the inheritance of voltinism is due only to the physiological influence of the foster mother, the term "maternal inheritance" employed by TOYAMA can not be correctly applied to the inheritance of voltinism. On the other hand, it was further observed that the voltinism, as well as all the other Mendelian characters, that is, the markings of larvae, the color of cocoons and so forth, of offspring (next generation) produced from the transplanted ovaries, were not at all influenced, there being nothing to show such an effect of the foster mother as GUTHRIE (1908) observed in similar experiments made with chickens.

New experiments, moreover, showed that the individuals reared from the eggs of transplanted ovaries have always higher vigor than those of the control, when the operation of ovarian transplantation is successfully

carried out. If the embryos developed out of the transplanted ovaries placed in different conditions are thus influenced, then it must be expected that the same effect will be seen in the crossing between two variants produced from one and the same breed under different environments. With this point of view, I have made further experiments in which I have also recognized the greater vigor of the crossing between hibernating (annual) and non-hibernating (biannual) variants produced from a bivoltine breed under different conditions, as compared with the original inbred strain. It is believed that these new experiments are not only practically important but that they also may throw some light on the cause of the vigor due to hybridization.

Before proceeding further, I should like to take this opportunity to tender my hearty thanks to Doctor C. ISIKAWA, for his valuable advice during the course of my present experiments. Thanks are due to Professor E. G. CONKLIN for his favor in looking through the manuscript.

VIGOR OF SILKWORMS PRODUCED FROM OVARIAN TRANSPLANTATION BETWEEN DIFFERENT BREEDS

Material and Method

As I have already stated in my previous report (1926) the technique of ovarian transplantation, I need not dwell on it here. To investigate the influence upon the vigor of the offspring resulting from the transplanted ovaries is more difficult than to observe directly the alternation of voltinism of the eggs from transplanted ovaries; because, in the former case, the host must be fully developed, more pains must be taken to prevent bleeding, and the offspring resulting from the transplant has to be well grown without suffering from the bad influence of the host.

For the purpose of making ovarian transplantation, the larvae of an early instar may be better, but it is not only impossible to operate on such very young larvae, but also impossible to use the largest fully-developed larvae of the last instar, because of the silk glands being squeezed out from the slit so that the operation cannot be carried out. Accordingly, the larvae at the beginning of the fifth instar (first and second day after the fourth moulting) were largely used in this case. As a matter of fact, the primordial egg-cells in the ovaries of larvae in such a stage are still so small that each contains only the nucleus and very little cytoplasm, but after the larvae have developed for about a week, the eggs rapidly enlarge, filling up with a large quantity of nutrients and cytoplasm, as reported by MATIDA (1926). Consequently, the larvae at the beginning of the fifth instar

are not unfavorable material for the purpose of investigating the physiological influence of the foster mother on the transplanted ovary.

Into the bodies of the pure breed, *Jap. No. 107*, a strong bivoltine race, from which the ovaries were removed, the ovaries of two other breeds were transplanted; one was the worm I termed cy strain, the reproductivity of which was extremely lowered by successive inbreeding since I first selected it in 1918, and the other the pure breed, *Jap. No. 105*, which showed no bad effect whatever of inbreeding for many years at the IMPERIAL SERICULTURAL EXPERIMENT STATION OF TOKYO.

Experimental results

In the experiments on ovarian transplantation we have as a rare occurrence a portion of the ovarian string duct remaining in the body of the host from which the ovary was removed which is connected with a point of the small part of the duct of the transplanted ovary, thereby forming an oviduct so favorably and completely connected with the ovarian string duct of the host that its development appeared normal, except that its four ovarian tubules were shorter in length, sometimes fewer in number and rarely contained any eggs. Accordingly, the eggs laid from the transplanted ovary in most cases were few in number. Into the bodies of the pure breed, *Jap. No. 107*, a bivoltine race, from which the ovaries were removed, the ovaries of the cy strain were transplanted. The results obtained from this experiment are given in table 1.

Table 1 indicates that the worms experimented upon showed increased weight and reproductiveness in spite of the shorter duration of larval life, as compared with those of the control. It is worth while to mention that the number of eggs in the experimented worms showed an increase by about 20 percent over the controls. Subsequently, into the bodies of the same breed as above described, the ovaries of the pure breed, *Jap. No. 105*, which showed no bad effects of inbreeding, were transplanted. These experimental results are given in table 2.

In the second table also, a tendency similar to that shown in table 1 is recognized, that is, the experimented worms showed greater vigor than the controls, though they showed a somewhat less degree of increased vigor than the cy strain showed. It is, however, noteworthy that the worms reared from the ovaries of a strain (table 1, cy worms) greatly weakened by successive inbreeding showed more strongly the influence of the host than did the pure breed (table 2, *Jap. No. 105* worms) which showed no bad effects of previous inbreeding.

Moreover, it is necessary to ascertain whether such influence of the fos-

TABLE 1
♀ (*Hos*—*Jap. No. 107*←*Ovaries—cy*) × ♂ *cy*.

SERIAL NUMBER	WEIGHT OF EACH WORM		TIME OF FEEDING DAYS—HOURS	NUMBER OF MOTHS	NUMBER OF EGGS			AVERAGE NUMBER OF EGGS	
	AFTER 3RD MOLTING GRAMS	AFTER 4TH MOLTING GRAMS			LAI D BY ALL MOTHS	REMAIN-ING IN BODIES	TOTAL	PRODUCED BY EACH MOTH	LAI D BY EACH MOTH
Experiment	32	0.103	0.485	6	3026	429	3455	575.83	504.33
	33	0.103	0.542	13	5906	775	6681	513.92	454.31
	34	0.100	0.482	11	4943	503	5446	495.09	449.36
	35	0.090	0.454	9	3957	381	4338	482.00	439.67
		(Average) 0.099	(Average) 0.491	(Total) 39	(Total) 17832	(Total) 2088	(Total) 19920	(Average) 513.462	(Average) 457.692
Control					(Standard deviation)			± 63.510	± 61.277
	37	0.098	0.472	21	7340	1163	8503	404.90	349.52
	38	0.085	0.410	10	4091	414	4505	450.50	409.10
	39	0.087	0.418	10	3762	691	4453	445.30	376.20
		(Average) 0.090	(Average) 0.434	(Total) 41	(Total) 15193	(Total) 2268	(Total) 17461	(Average) 428.049	(Average) 370.732
					(Standard deviation)			± 72.456	± 76.887

TABLE 2
♀ (Host—Jap. No. 107←Ovaris—Jap. No. 105) × ♂ Jap. No. 105.

	SERIAL NUMBER	WEIGHT OF EACH WORM		TIME OF FEEDING DAYS—HOURS	NUMBER OF MOTHS	NUMBER OF EGGS			AVERAGE NUMBER OF EGGS	
		AFTER 3RD MOULTING GRAMS	AFTER 4TH MOULTING GRAMS			LAI BY ALL MOTHS	REMAIN-ING IN BODIES	TOTAL	PRODUCED BY EACH MOTHS	LAI BY EACH MOTHS
Experiment	2	0.154	0.928	31-19	16	7236	746	7982	498.88	452.25
	3	0.157	0.908	31-0	10	4999	584	5583	558.30	499.90
	4	0.147	0.833	31-0	12	5994	644	6638	553.17	499.50
	6	0.167	0.843	32-0	34	16143	1319	17462	513.59	474.80
	7	0.162	0.850	31-6	13	6138	431	6569	505.31	472.15
	11	0.167	0.780	32-12	63	29560	2566	32126	509.94	469.20
	15	0.159	0.792	32-4	15	6930	762	7692	512.80	462.00
	16	0.157	0.748	32-12	21	10273	1291	11564	550.67	489.19
	19	0.152	0.795	32-0	17	7735	704	8439	496.41	455.00
		(Average) 0.158	(Average) 0.830	(Average) 31-19	(Total) 201	(Total) 95008	(Total) 9047	(Total) 104055	(Average) 519.527	(Average) 473.383
Control						(Standard deviation)			± 60.119	± 57.035
	21	0.140	0.741	32-0	60	27179	1255	28434	473.90	452.98
	22	0.150	0.672	32-12	70	31617	1703	33320	576.00	451.67
	23	0.134	0.698	31-10	50	22693	1360	24053	481.06	453.86
	24	0.162	0.735	31-19	49	19781	1890	21671	442.27	403.69
	25	0.160	0.780	32-12	36	15293	1469	16762	465.61	424.86
		(Average) 0.149	(Average) 0.725	(Average) 32-1	(Total) 265	(Total) 116563	(Total) 7677	(Total) 124240	(Average) 468.019	(Average) 440.094
						(Standard deviation)			± 74.188	± 77.355

ter mother upon the offspring could be carried over to succeeding generations. For that purpose, the vigorous lines of worms in the above two tables were selected out of those thus made vigorous, and were reared in the autumn to compare with the worms of the original line. The results obtained were as follows:

TABLE 3

Rearing results of the next generation of the cy strain used in the experiment of table 1.

	SERIAL NUMBER	WEIGHT OF EACH WORM AFTER 4TH MOULTING (GRAMS)	TIME OF FEEDING DAYS-HOURS	NUMBER OF MOTHS	NUMBER OF EGGS	
					LAID BY ALL MOTHS	LAID BY EACH MOTH
Experiment	140	0.392	24-23	11	3641	331.00
	141	0.366	26-15	8	2828	353.50
	142	0.367	26-21	8	2629	328.63
	144	0.390	24-21	20	6405	320.25
	146	0.360	24-15	10	2844	284.40
	147	0.343	24-23	17	5643	331.94
		(Average) 0.370	(Average) 25-12	(Total) 74	(Total) 23990	(Average) 324.324
Standard deviation ± 64.981						
Control	151	0.356	26-23	8	2529	316.13
	152	0.389	24-23	8	2547	318.38
	153	0.360	26-21	10	3016	301.60
		(Average) 0.368	(Average) 26-6	(Total) 26	(Total) 8092	(Average) 309.615
	Standard deviation ± 66.534					

In table 3, further influence on the next generation was recognized, though in a less degree. On the other hand, table 4 showed no trace whatever of any influence exerted on the second generation by the original transplantation.

VIGOR OF SILKWORMS OBTAINED FROM THE CROSSING OF TWO VARIANTS BRED UNDER DIFFERENT ENVIRONMENTS

The results obtained from the foregoing experiments led me to try again the following experiment. From the eggs of a bivoltine breed, worms of 2 groups were hatched out by subjecting them to the conditions induced by different environments; 1 was made completely univoltine by the application of a high temperature of over 25°C during the incubation of the em-

bryonal stages, while the other became bivoltine under a temperature less than 15°C. As I have already reported, we ascertained that the alternation of voltinism was caused by the local condition, and each individual bearing different voltinism did not contain the same blood as regards the determination of voltinism, that is, the univoltine individual tended to secrete the voltinism-determiner (so-called inhibitory substance of WATANABE) from the post-embryonal somatic cells by the stimulus of a high tem-

TABLE 4

Rearing results of the next generation of the breed Jap. No. 105 used in the experiment of table 1.

	SERIAL NUMBER	WEIGHT OF EACH WORM AFTER 4TH MOULTING (GRAMS)	TIME OF FEEDING	NUMBER OF MOTHS	NUMBER OF EGGS	
			DAYS-HOURS		LAID BY ALL MOTHS	LAID BY EACH MOTHS
Experiment	130	0.664	25-20	27	9559	354.04
	132	0.658	25-20	16	6704	419.00
	136	0.664	25-20	27	10902	403.77
	137	0.632	25-20	30	12276	409.20
		(Average) 0.655	(Average) 25-20	(Total) 100	(Total) 39441	(Average) 394.250
Standard deviation ± 64.600						
Control	117	0.645	25-20	35	13976	399.31
	118	0.682	25-20	26	10205	392.50
	119	0.661	25-20	16	6441	402.56
	120	0.681	25-20	42	16730	398.33
		(Average) 0.667	(Average) 25-20	(Total) 119	(Total) 47352	(Average) 398.529
Standard deviation ± 69.981						

perature and to transmit it into egg-cells in their maturation, thus causing them to hibernate, while the bivoltine individuals which developed at a low temperature did not secrete such a substance and consequently their eggs did not hibernate. It was interesting to note from the foregoing experiments the results of rearing worms obtained from the crossing of 2 such variants produced from one and the same breed under different temperatures, as compared with the pure original breed.

Material and method

One batch of eggs laid by a moth of the pure bivoltine race was divided into 2 equal parts; 1 was incubated and subjected to the high temperature

(25°C) in order to produce the moths laying hibernating eggs, the other to the low temperature (15°C) to make the moths lay non-hibernating eggs. Thus, these 2 groups of worms were obtained for the purpose of breeding one with the other reciprocally. The hibernating eggs were hatched out artificially in the autumn for the purpose of rearing them together with the non-hibernating eggs which were hatched out naturally. The material used for these experiments was the pure bivoltine breed, *Jap. No. 105*.

Experimental results

The rearing results obtained from the crossing of 2 variants bred under different environments, as above stated, will be shown in the following 2 tables.

TABLE 5

Rearing results of worms hatched artificially from hibernating eggs.

	SERIAL NUMBER	WEIGHT OF EACH WORM AFTER 4TH MOULTING (GRAMS)	TIME OF FEEDING	NUMBER OF MOTHS	NUMBER OF EGGS	
			DAYS-HOURS		LAID BY ALL MOTHS	LAID BY EACH MOTH
Experiment ♀ hibernating × ♂ non-hi- bernating	109	0.712	24-19	43	20391	474.21
	110	0.772	24-23	130	60528	465.60
	111	0.784	24-23	111	51452	463.55
	112	0.662	25-19	127	56144	442.08
		(Average) 0.733	(Average) 25- 3	(Total) 411	(Total) 188515	(Average) 459.124
Standard deviation ± 61.920						
Control ♀ hi- bernating × ♂ hibernating	101	0.713	25-14	150	61520	410.13
	102	0.715	24-22	127	57816	455.24
	103	0.730	25- 0	103	45087	437.74
	104	0.743	24-23	119	52201	438.66
		(Average) 0.725	(Average) 25- 3	(Total) 499	(Total) 216624	(Average) 434.369
Standard deviation ± 72.658						

In these two tables also, the crossings between 2 characters produced under different environments showed a shorter duration of larval life, a somewhat greater weight and higher reproductivity, as I presumed, thus showing higher vigor as the previous experiments indicated.

The sericulturist knows by experience that when 2 variants of univoltine

and bivoltine worms are produced from a bivoltine breed and reared at the same time in the autumn, the latter should show a somewhat greater vigor than the former. In this case also, the rearing results of the control in table 6 (bivoltine worms) showed a somewhat greater vigor than those of the control in table 5 (univoltine worms). These results were very likely due

TABLE 6
Rearing results of worms hatched naturally from non-hibernating eggs.

	SERIAL NUMBER	WEIGHT OF EACH WORM AFTER 4TH MOULTING (GRAMS)	TIME OF FEEDING	NUMBER OF MOTHS	NUMBER OF EGGS	
			DAYS-HOURS		LAID BY ALL MOTHS	LAID BY EACH MOTHE
Experiment ♀ non-hiber- nating ♂hi- bernating	113	0.715	24-19	103	48082	466.82
	114	0.771	25- 0	170	84038	494.34
	115	0.739	25- 0	131	63187	482.34
	116	0.740	26- 0	127	62915	495.39
		(Average) 0.741	(Average) 25- 5	(Total) 531	(Total) 258222	(Average) 486.676
Standard deviation ± 61.233						
Control ♀ non-hibernat- ing ♂non-hi- bernating	105	0.688	25-19	96	39410	410.52
	106	0.762	25-14	189	87008	460.36
	107	0.737	26- 0	148	66988	452.62
	108	0.752	26- 0	68	32023	470.93
		(Average) 0.735	(Average) 25-19	(Total) 501	(Total) 225429	(Average) 449.551
Standard deviation ± 71.786						

to the following reasons: the bivoltine worms were reared in the autumn under a more favorable environment than the univoltine ones reared at the same time under unfavorable conditions. Accordingly, it might be considered that the experiment shown in table 5 made it appear that the greater vigor of the crossing was caused by the vigor of the bivoltine spermatozoa which were much stronger under a favorable condition than those of the univoltine. If this were true, such spermatozoa influence ought to be also recognized in the experimented worms of table 6. But, on the contrary, we noted that the worms crossed by fertilization with univoltine spermatozoa which became weaker under unfavorable conditions than the bivoltine, nevertheless showed more vigor than the original bivoltine worms. Consequently, it seems more reasonable to consider the vigor of worms as the

result of the surrounding conditions of the embryos, that is, of the ooplasm and nutrients of egg-cells, rather than as proceeding from the direct influence of spermatozoa. When the univoltine worms are inbred, the spermatozoon which entered into the egg-cell is surrounded by its own univoltine ooplasm and nutrients, likewise the bivoltine spermatozoon by its own bivoltine surroundings. On the other hand, in the cross-breeding between these 2 variants, such a spermatozoon is to be controlled by the ooplasm and nutrients which were not in their own normal surroundings. In other words, the former case of inbreeding showed that the nuclei and cytoplasm were not at all modified by any external influence, but in the latter case of the crossing of 2 variants, only the somatic cells of the embryo were modified by the cytoplasm of spermatozoon, and the embryo was physiologically influenced by the blood of the foster mother which, in turn, was changed by the temperature. Consequently, such an embryo would develop in ooplasm and nutrients, forming an abnormal environment for that embryo.

Judging from the results above obtained by experiments on insects, I felt confident that some influence of the cytoplasm of the embryo sac upon the embryos could also be seen in plants. At the suggestion of the author, similar experiments on plants have been carried out by RYI at the GOVERNMENT AGRICULTURAL EXPERIMENT STATION OF CHOSŌN. These results will be briefly given in this paper with a view to extending to plants my conclusions here stated. Two groups of Eckendorfer (a pure breed of barley), one of which was obtained by sowing in the autumn and the other in the early spring, were crossed reciprocally at flowering time in the late spring. Then these two crossings were sown at the same time in the autumn with the original self-fertilized. The results obtained in the summer of 1927 were as follows:

TABLE 7

Results of crossings of 2 groups produced from Eckendorfer barley under different environments.

EXPERIMENT AND CONTROL	NUMBER OF STOCKS EXAMINED	DATE OF HEADING	NUMBER OF TILLER	LENGTH			WEIGHT OF PANICLE GRAMS
				STOCK CM	PANICLE CM	AWN CM	
Self-fertilized	108	V-28.6	6.7	93.03	8.79	12.73	16.13
Crossed in different stocks	25	V-28.4	9.6	95.15	9.70	12.73	23.63
♀ Sowed in spring × ♂ Sowed in autumn	10	V-28.6	10.0	92.73	10.00	12.42	26.63
♀ Sowed in autumn × ♂ Sowed in spring	7	V-28.8	12.1	95.15	9.70	12.73	23.63

As shown in table 7, the crossings between 2 groups produced from a pure barley by sowing it under different conditions showed distinctly higher vigor than the original line self-fertilized. It is most important and interesting to note that a tendency similar to that shown in the previous experiments was also proved in these plants. Moreover, DARWIN (1876) had already observed that cross-fertilization between different flowers in the same individual was better than self-fertilization, and lately YASUDA (1927) proved that in fertility of *Petunia violacea* the greater the difference of physiological conditions of the flowers the better. These observations agree with the results of the experiments carried out by myself and described here.

GENERAL CONSIDERATION

Influence of the ooplasm and nutrients upon the embryo

Whether some conditions of the parents do exert any influence upon the offspring or not is so very important for practical sericulture that it has been repeatedly discussed by many interested authors. The results of the experiments carried out by myself and described here leave no doubt of the existence of such an influence. It is, however, to be expected that the results differ in accordance with the degree of environmental stimuli exerted upon the parents as well as with the difference in environment during the rearing of the offspring. When the conditions in which the worms are reared in the next generation are favorable for growth, it might happen that the influence of the preceding generation might not appear in the least on the worms of the subsequent generation. On the contrary, when the rearing conditions are unfavorable, even a low degree of influence exerted on the preceding generation might appear in the succeeding generation.

But how such a phenomenon occurs is not yet clear. Judging from the results here stated, I presume that it is principally due to the action of the ooplasm and nutrients of the egg-cells which are affected by the blood of the foster mother; this is the case with the very large egg-cells as compared with the spermatozoa of silkworms. As above stated, since hibernating or non-hibernating eggs could be produced by a change in the blood of the foster mother from a bivoltine breed, it is quite probable that, by the alteration of environment, we could also change the blood of the mother, as well as in the case of the alternation of voltinism. If this is assumed, then it could naturally be considered that the embryos must receive some influence through the ooplasm and nutrients of eggs which are controlled by the blood, though the spermatozoa, in some instances, might to some

extent be effective as recognized by the influence of alcohol upon the spermatozoa (STOCKARD 1913, STOCKARD and PAPANICOLAOU 1918). The results of first experiments carried out by ovarian transplantation between the different breeds showed obviously that the embryos were influenced by the ooplasm and nutrients produced by the different blood of the host.

The maintenance of vigor by the worms after they had passed through the embryonal stages, that is, after they had passed out of the environmental influence of the host, could be considered as due to "after-reaction" of the stimulus on the egg-cells.

Vigor resulting from hybridization

The high vigor of the F_1 hybrid and the lowering of this in the F_2 have been observed by many biologists (KOELREUTER, DARWIN, WEISMANN, CASTLE and others) and the cause of it is attributed to the dominant allelomorphs of the forms crossed (CASTLE, SHULL, EAST, KEEBLE, PELLEW, JONES and others). I do not oppose this opinion, but I can not quite agree that the source of the vigor of the F_1 hybrids is found exclusively in the reaction of these genetic factors. If we should try to explain the results of the foregoing experiments by diagrammatic figures and compare them with the F_1 hybrid, the results would be as shown on page 201.

Figure 1 indicates a case of normal inbreeding representing the crossing of a pure breed in which the nuclei a and cytoplasm A of egg-cell and spermatozoon a are not at all modified by any external influence, the embryo aa thus developed typically. Figure 2 indicates a case of ovarian transplantation representing the ooplasm and nutrients A in which the embryo bb is developed, thus giving a quite different environment to the embryo. Figure 3 shows the crossing between 2 variants produced by environmental changes. In this case, the crossing is made between the univoltine and bivoltine variants produced from one and the same bivoltine breed. The symbol a' indicates a spermatozoon, of which the nucleus is not influenced by the external conditions, but of which the cytoplasm is quite modified by the temperature. Accordingly, such a spermatozoon is naturally adapted to the ooplasm and nutrients A' of its own normal surroundings. But it must, in this case, fertilize an egg-cell which has a different condition of ooplasm and nutrients A . It may be said in this connection that in fertilization of the germ cells of animals, a part or most of the tail (portion of cytoplasm) of the spermatozoon enters into the egg-cell along with some mitochondria, as already reported by SOBOTTA, MEVES, VAN DER STRICHT, KOSTANECKI, WIERZEJSKI and others, so it leaves no room for doubt that in insects the embryo is influenced by the cytoplasm of the spermatozoon

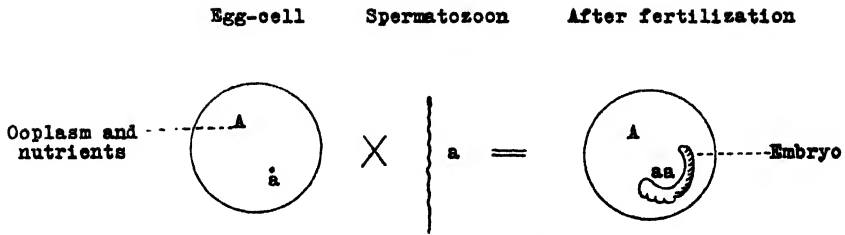


FIGURE 1.—The case of inbreeding.

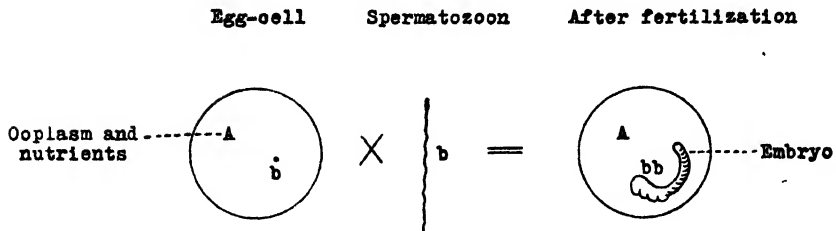


FIGURE 2.—The case of ovarian transplantation.

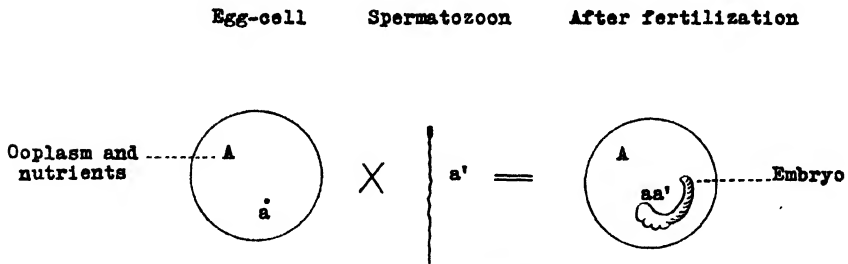


FIGURE 3.—The case of crossing two variants.

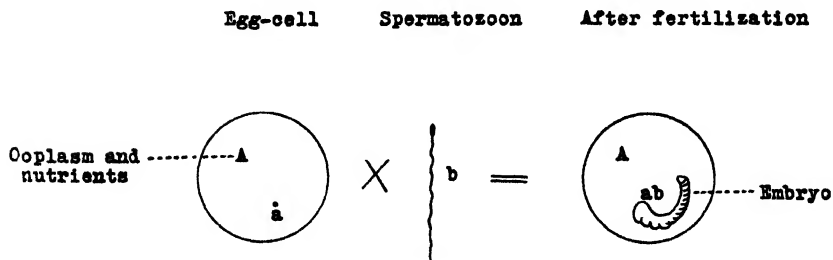


FIGURE 4.—The case of hybridization.

which is affected by its environment. Thus, figure 3 indicates that only the somatic cells of the embryo are modified by the cytoplasm of the spermatozoon, but that its genetic factors are not influenced. Accordingly, such an embryo aa' must develop in the environment A which is different from that for which the embryo aa' is adapted, so that this is rather similar to the case indicated in figure 2 (ovarian transplantation), in spite of its resemblance to the case of figure 1 (inbreeding). Lastly, figure 4 represents the usual case of the F_1 hybrid, that is, the embryo ab produced from a heterozygote developing in the ooplasm and nutrients of A. This case rather resembles the cases indicated in figures 2 and 3. If we, therefore, recognize the influence of ooplasm and nutrients upon the embryo as shown in figures 2 and 3, we must also recognize a similar tendency in the case of the F_1 hybrid. Moreover, we can consider that the influence of the hybrid vigor generated by the ooplasm and nutrients is clearly seen in the F_1 generation as shown in table 4, but may scarcely extend to the F_2 hybrid, though we have a rare instance where it may be in a less degree extended to the succeeding generation as we see in table 3. Consequently, it is suggested that the cause of the high vigor of the F_1 hybrid and the lowering of this in the F_2 cannot be looked upon as a result of the reaction of the heterozygotes only, as so many geneticists are inclined to assume.

The maintenance of the influence caused by an environment on the post-embryonal stages of the hybrid as well as on the worms of later generation should also be considered as a case of "after-reaction." Hence, there is nothing against the assumption that the high vigor of hybridization is due, in addition to the reaction of the dominant heterozygotes, to the stimulus exerted upon the developing embryos by the ooplasm and nutrients of the egg-cells. For this reason, strictly speaking, the term "heterosis" should not be limited to cases of hybrid vigor.

SUMMARY

1. In the ovarian transplantation between different breeds of silkworms, the individuals reared from an ovary transplanted into a different breed always showed a shorter duration of larval life, a greater weight and higher reproductivity than those of the control, especially in the case where the operation of transplantation was successfully carried out and the individual into which the ovaries were transplanted was fully developed and was not physiologically injured by bleeding from the slit made when it was operated upon.

2. The worms reared from the transplanted ovaries of a pure breed greatly weakened by successive inbreeding showed more strongly the in-

vigorating influence of the host than did the other pure breed which showed no bad effects of inbreeding.

3. Crosses between univoltine and bivoltine worms produced from a pure bivoltine stock under different environments also showed higher vigor than those of the original breed, as the above experiments indicated. And such phenomena as these in insects have also been observed in the crossing of two groups produced from a pure barley.

4. The question as to whether some conditions of the parents do exert any influence upon the offspring or not depends upon the degree of environmental stimulus exerted upon the parents as well as upon the difference of environment during the rearing of the offspring. And, in silkworms, the egg-cells of which are much larger than the spermatozoa, such an influence upon the offspring is principally due to the after-reaction of the stimulus of the ooplasm and nutrients controlled by the blood of the foster mother though in rare instances it is due to the spermatozoa.

5. It is reasonable to suggest that the cause of the high vigor of hybridization is due, not only to the reaction of the dominant allelomorphs, as so many geneticists are inclined to assume, but also to the after-reaction of the stimulus exerted upon the developing embryos by the ooplasm and nutrients of the egg-cells. Accordingly, the term "heterosis," strictly speaking, should not be limited to cases of vigor due to hybridization.

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CROSSING OVER IN THE THIRD CHROMOSOMES OF TRIPLOIDS OF *DROSOPHILA MELANOGASTER**

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INTRODUCTION

Upon *a priori* grounds it has long been assumed that any crossover involves at least two phenomena: (1) a rotation or overlapping of chromosomes or of their derivative strands, and (2) a breaking and reunion at one or more loci of these strands. (For a recent discussion see BELLING 1927.) It is known in *Drosophila* that the segmental exchange does not occur in males and that it takes place in diploid females at the four-strand stage (BRIDGES 1916, ANDERSON 1925, L. V. MORGAN 1925, STURTEVANT unpublished) and in triploid females at the corresponding six-strand stage (BRIDGES and ANDERSON 1925). Further evidence indicates that the process occurs in early oöcytes before the first maturation division (PLOUGH 1917, 1921; GOWEN 1929). The amount of double crossing over is dependent upon the distance between the loci involved and varies with the region of the chromosome under observation (BRIDGES and MORGAN 1923). Crossing over may be partially or completely inhibited by "crossover reducers" (STURTEVANT 1919, GOWEN 1928, etc.) some of

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¹ Work completed while the writer was National Research Fellow in Zoology, COLUMBIA UNIVERSITY.

which have been shown to be inverted sections of chromosome (STURTEVANT 1926).

A number of recent results indicate a correlation between the effects of various agents and the distance of the region involved from the spindle-fibre attachment. Using young females as contrasted with older females, or subjecting females to temperatures greater or less than about 25°C, or to X-rays, results in a considerable increase in crossing over in the region of spindle-fibre attachment (BRIDGES 1915, 1927; PLOUGH 1917, 1921; MAVOR 1923; MAVOR and SVENSON 1924; MULLER 1925, 1926; STERN 1926). This is undoubtedly true of the V-shaped second and third chromosomes in which the attachment is at the apex of the V, and is probably true also of the rod-shaped X chromosome in which the attachment is terminal and at the right end of the chromosome map. The fourth chromosome has not been tested.

Whatever the mechanical factors at work, it would seem that a substitution of six sets of crossover strands in the triploid for the four sets ordinarily present in the diploid might throw some light upon the process of crossing over. However, besides the mere substitution of a greater number of chromosome strands, triploidy involves changes in the general relationships within the cell, and, in particular, possible changes in the ratio of chromatin to cytoplasm as regards surface and volume. Such changes in themselves might be responsible for the crossover variations found. This question will be considered later.

One of the main difficulties encountered in the analysis of triploid data relates to the possibility of crossing over of sister strands (that is, the two strands resulting from the splitting of a single chromosome). Crossing over is ordinarily measured by the percentage of crossed-over chromosomes detected. But in both diploid and triploid, crossing over between sister strands, if it occurs, will remain undetected. If it is as likely to occur between sister strands as between non-sister strands, then in the diploid in any given region the undetected crossing over would be $\frac{1}{2}$ the actual total crossing over, that is, the ordinary "crossover" values would represent $\frac{2}{3}$ the actual physical crossing over. But in the triploid, since six strands are involved, only $\frac{1}{3}$ the total crossing over would be undetected — in other words, the detected "crossing over" would represent $\frac{2}{3}$ the total crossing over.

A simple correction may, of course, be made for sister-strand crossing over in comparing diploid with triploid, but it rests upon the assumption of free sister-strand interchange. It was expected, as a matter of fact,

that the possibility of such interchange could be investigated by means of data from triploids. Unfortunately, as BRIDGES and ANDERSON (1925) also found, the difference between triploid and diploid shows such great variation in different regions of the chromosome that any effect to which sister-strand interchange may give rise is completely masked. Corrected values will be tabulated whenever it seems advisable. These corrections are superfluous, if, as the recent paper of STURTEVANT (1928) indicates, there is no sister-strand crossing over. He finds that controlled cases of reversion of bar eye to round are always accompanied by detected crossing over. If sister-strand crossing over occurred at all in such crosses, it should have given many more apparent non-crossover reversions than can be explained on the basis of experimental error. On the other hand it is possible that bar reversion represents a special case, and if so there is no way of knowing at present whether or not sister-strand interchange takes place. However, as will be shown later (page 236), the conclusions of the present paper in regard to the differences between crossing over in triploid and diploid remain unaltered, even if sister strands cross over as freely as non-sister strands.

CROSSES IN WHICH THE THREE THIRD CHROMOSOMES WERE MARKED

Technically the study of crossing over in triploids is made difficult by a number of circumstances. Thus, relatively few offspring are obtained, and these offspring are of various types in regard to their chromosome balance. They include approximately: 3.9 percent triploid daughters, 46.8 percent diploid daughters, 6.0 percent diploid sons, 36.9 percent intersexes, 1.3 percent superfemales, and 5.2 percent supermales (see BRIDGES and ANDERSON 1925). The facts that some expected chromosome combinations do not appear at all (for example, individuals diploid except for the presence of three second or third chromosomes) and that the percentages of the types which do appear are very different from expectation, are believed to depend upon viability differences during development.

In the investigations of BRIDGES and ANDERSON on crossing over in the X chromosome advantage was taken of the fact that of the 46.8 percent diploid daughters produced, practically all (that is, 41.1 percent of the total offspring) represent exceptions which receive a Y chromosome from the father, the two X chromosomes coming from the triploid mother. Data on crossing over for this chromosome may then be obtained by genetic tests of the composition of the exceptional daughters.

But the corresponding exceptions for the two larger autosomes do not appear, and this technique is therefore not applicable in the study of chromosome III. The diploid sons and daughters have each received one third chromosome from the triploid mother and one from the diploid father. Crossing over is then measured directly by the relative proportions of the diploid types emerging from the cross.

In the crosses first successfully utilized all three third chromosomes were marked according to the scheme previously adopted by BRIDGES and ANDERSON. A series of mutant loci were chosen, such that very near each locus another favorable mutant gene is known for each of the points chosen; the two genes and the normal locus of the first were introduced into the triploid, one in each of the three chromosomes, and were treated virtually as a series of allelomorphs. Actually one is non-allelomorphic but is so close to the other locus that crossing over between them is relatively negligible. Because the characters which it seemed advisable to use interfere to a certain extent with each other, it was necessary, in covering the chromosome, to make use of two crosses instead of one. These crosses are as follows:

$$(1) \quad \begin{array}{ccccccccc} + & D & m_a & S_b & + & & & & \\ \hline s_e & M_h & + & + & H & & & & \\ \hline h & + & c_u & b_x & e^s & & & & \end{array} \quad \text{♀} \times s_e \ h \ m_a \ c_u \ b_x \ e^s \ \sigma^7.$$

$$(2) \quad \begin{array}{ccccccc} + & H & + & & & & \\ \hline S_b & + & M & & & & \\ \hline b_x & e^s & c_a & & & & \end{array} \quad \text{♀} \times b_x \ e^s \ c_a \ \sigma^7.$$

The symbols stand for the following third chromosome mutants, the genes having the positions indicated on the standard diploid map: s_e , sepia at 26.0 and h , hairy one-half unit to the right of sepia; M_h , minute-h at 40.1 and D , dichaete at 40.4; m_a , maroon at 49.7 and c_u , curled at 50.0; S_b , stubble at 58.2 and b_x , bithorax at 58.7; H , hairless at 69.5 and e^s , sooty at 70.7; c_a , claret at 100.7 and M , minute at 101.0.

Of the seven special stocks needed for the work, only hairless was already in existence; it was necessary therefore to synthesize the others. Each stock was derived from a single chromosome which was then (excepting $h \ c_u \ b_x \ e^s$ and $s_e \ h \ m_a \ c_u \ b_x \ e^s$) kept intact by being carried over the

$IC_L C_R$ combination which prevents crossing over. Each was then inbred for several generations before being used.

The appearance of the triploid with a single dose of the mutant genes involved is of some interest. As would be expected, the recessives do not become manifest. A dominant, on the other hand, may or may not have an observable effect. Thus minute and minute-h do not appear in single dose; hairless however has a distinct effect but is less extreme than in the diploid. And, finally, the manifestation of dichaete and stubble does not differ appreciably in triploid and diploid.

There are certain pronounced difficulties connected with the use of the stocks. In the first place it is impossible to distinguish between sepia and the sepia-maroon double recessive. This makes it necessary either to introduce a third eye color which behaves as a sensitizer and thus aids in the separation of the various eye-color types, or to test genetically all sepia-appearing diploid offspring (a third of all the diploids) for the presence of maroon. The genetic test was used until late in the work, when it was discovered that vermillion (a sex-linked recessive) behaves as a sensitizer. At this stage all the stocks were accordingly made homozygous for vermillion. But even in the presence of vermillion, the vermillion-sepia eye (a brassy orange color) and the vermillion-sepia-maroon eye (lemon yellow) become indistinguishable after the first half-day. It is necessary then to classify the flies before they are more than about ten hours old.

In the second place most of the dominants used are lethal in double dose in triploids and intersexes. This is true of minute, minute-h and dichaete; double hairless occasionally survives but is very extreme in appearance. Although double stubble was not detected in these experiments, it frequently survives, according to the unpublished data of SCHULTZ. In these crosses, due to the lethal effects, it is not possible to determine the position of the spindle-fibre attachment, for this determination depends upon the relative numbers of equational exceptions at the various loci (see page 245). There are further difficulties, in particular those connected with obtaining triploids of the proper composition to test, but these need not be discussed here.

The diploid controls include the following crosses from the same stocks used for the triploid crosses:

$$(1) \quad \frac{s_e \quad M_h \quad + \quad + \quad H}{h \quad + \quad c_u \quad b_z \quad e^+} \quad \text{♀} \times s_e \quad h \quad m_a \quad c_u \quad b_z \quad e^+ \quad \text{♂}.$$

$$(2) \quad \frac{\begin{array}{ccccc} + & D & m_a & S_b & + \\ h & + & c_u & b_x & e^s \end{array}}{\quad} \text{♀} \times s_s h m_a c_u b_x e^s \text{♂}.$$

$$(3) \quad \frac{\begin{array}{ccc} + & H & + \\ b_x & e^s & c_a \end{array}}{\quad} \text{♀} \times b_x e^s c_a \text{♂}.$$

$$(4) \quad \frac{\begin{array}{ccc} + & H & + \\ S_b & + & M \end{array}}{\quad} \text{♀} \times b_x e^s c_a \text{♂}.$$

The actual procedure was to isolate a diploid or triploid virgin of the required composition and to put her into a small vial with five males from the proper tester stock. These six flies were allowed to remain in the vial one day, after which they were transferred to the usual half-pint culture bottle provided with the cornmeal-molasses-agar medium. After five days in the first culture bottle they were transferred to a second new culture bottle and remained there five days. In the early stages of the work a transfer was then made to a third culture bottle, but it soon became obvious that triploid females give practically no offspring in these third cultures; and all females were thereafter discarded after the first eleven days of their life. These transfers were, of course, made in order to detect any gross variations with age. A more delicate test of the effect of age did not seem advisable.

The data for these triploid and diploid crosses appear in tables 1, 2, 3, and 4. In these tables the numerals "I" and "II" refer to the first and second cultures described above, that is "I" includes flies hatched in the first cultures from eggs laid on the second to the sixth days inclusive of the mother's life, and "II" includes flies from the second cultures from eggs laid on the seventh to the eleventh days.

The data from triploid mothers are given in full in tables 1 and 3. Since the control data (tables 2 and 4) are ordinary diploid data it was not considered necessary to present them in similar space-consuming form. The short laying period and the care taken of the culture bottles insured a reasonable equality of contrary classes; no viability differences appeared which were great enough to necessitate corrections.

In the data from triploids of the type $a_1a_2a_3 \dots / b_1b_2b_3 \dots / c_1c_2c_3 \dots$ two types of double crossovers are to be distinguished. The first are the so-called "recurrent" doubles in which the same two chromosomes are involved in the two succeeding crossings over (for example $a_1b_2a_3$); the

TABLE 1

$+ D m_a S_b +$
 Offspring of the cross $\frac{s_e M_h ++ H}{h + c_u b_x e^e} \quad \text{♀} \times s_e h m_a c_u b_x e^e \text{♂}$. *R* indicates recurrent double crossovers;
P, progressive double crossovers.

		I; 21 CULTURES		II; 18 CULTURES			
Triploids		29		19			
♀ Interseses		186		129			
♂ Interseses		19		19			
Superfemales		..		1			
Supermales		..		2			
Diploids							
Type	Classes	♀	♂	Total	♀	♂	Total
0	<i>D m_a S_b</i>	103	15	340	68	18	225
	<i>s_e M_h H</i>	95	14		67	5	
	<i>h c_u b_x e^a</i>	93	20		62	5	
1	<i>s_e D m_a S_b</i>	9	..	47	7	..	37
	<i>M_h H</i>	7	.		5	..	
	<i>h D m_a S_b</i>	8	3		7	1	
	<i>c_u b_x e^a</i>	6	..		3	.	
	<i>s_e c_u b_x e^a</i>	9	.		6	2	
	<i>h M_h H</i>	5	..		6	..	
2	<i>D H</i>	12	1	110	8	2	61
	<i>s_e M_h m_a S_b</i>	9	3		4	..	
	<i>D c_u b_x e^a</i>	15	1		15	1	
	<i>h m_a S_b</i>	18	4		9	1	
	<i>h H</i>	24	..		12	..	
	<i>s_e M_h c_u b_x e^a</i>	22	1		7	2	

TABLE 1 (*continued*)

Diploids		I; 21 cultures			II; 18 cultures			
Type	Classes	♀	♂	Total	♀	♂	Total	
3	<i>D m_a H</i>	8	1	40	7	1	29	
	<i>s_r M_h S_b</i>	5	2		4	1		
	<i>D m_a b_x e^a</i>	7	2		2	..		
	<i>h c_u S_b</i>	6	1		6	1		
	<i>h c_u H</i>	4	1		3	..		
	<i>s_e M_h b_x e^a</i>	2	1		2	2		
4	<i>D m_a S_b H</i>	2	..	29	5	..	17	
	<i>s_r M_h</i>	4	1		1	1		
	<i>D m_a S_b e^a</i>	7	1		1	.		
	<i>h c_u b_x</i>	2	..		4	..		
	<i>h c_u b_x H</i>	7	1		1	..		
	<i>s_e M_h e^a</i>	4	..		3	1		
1,2	R	<i>s_r D H</i>	2	..	16	1	.	5
		<i>M_h m_a S_b</i>	4	
		<i>m_a S_b</i>	2	
		<i>h D c_u b_x e^a</i>	
		<i>s_e H</i>	
		<i>h M_h c_u b_x e^a</i>	
	P	<i>H</i>	1	..		1	..	
		<i>M_h c_u b_x e^a</i>	2	
		<i>s_e m_a S_b</i>	1	..		1 ^u	..	
		<i>s_e D c_u b_x e^a</i>	
		<i>h D H</i>	3	..		1	..	
		<i>h M_h m_a S_b</i>	1	1	

TABLE 1 (continued)

Diploids			I; 21 CULTURES			II; 18 CULTURES		
Type	Classes		♀	♂	Total	♀	♂	Total
1,3	R	$s_a D m_a H$	1	..	7	4
		$M_h S_b$	1	
		$h D m_a b_x e^a$	
		$c_u S_b$	1	
		$s_a c_u H$	1	
		$h M_h b_x e^a$	
	P	$c_u H$		1	..	
		$M_h b_x e^a$	1	.		1	..	
		$s_a D m_a b_x e^a$	
		$s_a c_u S_b$	
		$h D m_a H$	1	.		..	1	
		$h M_h S_b$	1	..		1	..	
1,4	R	$s_a D m_a S H$.	..	4	1	..	3
		M_h	
		$c_u b_x$	
		$h D m_a S_b e^a$	
		$s_a c_u b_x H$	
		$h M_h e^a$	
	P	$c_u b_x H$	1	
		$M_h e^a$	1	..		1	.	
		$s_a D m_a S_b e^a$		1	..	
		$s_a c_u b_x$	
		$h D m_a S_b H$	1	
		$h M_h$	1	

TABLE 1 (continued)

Diploids			I; 21 CULTURES			II; 18 CULTURES		
Type	Class		♀	♂	Total	♀	♂	Total
2,3	R	<i>D S_b</i>	3	1	22	1	..	5
		<i>s_e M_h m_a H</i>	1	
		<i>D c_u S_b</i>	2	
		<i>h m_a b_x c^e</i>	2	..		1	..	
		<i>h b_x c^e</i>	2	
		<i>s_e M_h c_u H</i>	1	
	P	<i>D b_x c^e</i>	3	
		<i>D c_u H</i>	..	1		
		<i>s_e M_h m_a b_x c^e</i>		1	..	
		<i>s_e M_h c_u S_b</i>	1	..		1	..	
		<i>h m_a H</i>	1	
		<i>h S_b</i>	3	1		1	.	
2,4	R	<i>D</i>	1	..	10	1	..	2
		<i>s_e M_h m_a S_b H</i>	
		<i>D c_u b_x</i>	
		<i>h m_a S_b c^e</i>	2	..		1	..	
		<i>h c^e</i>	1	
		<i>s_e M_h c_u b_x H</i>	
	P	<i>D c^e</i>	
		<i>D c_u b_x H</i>	2	
		<i>s_e M_h m_a S_b c^e</i>	2	
		<i>s_e M_h c_u b_x</i>	2	
		<i>h m_a S_b H</i>	
		<i>h</i>	

TABLE 1 (continued)

Diploids			I; 21 CULTURES			II; 18 CULTURES		
Type	Class		♀	♂	Total	♀	♂	Total
3,4	R	<i>D m_a</i>	5	1
		<i>s_e M_h S_b H</i>	
		<i>D m_a b_z</i>	
		<i>h c_u S_b c^e</i>	3	
		<i>h c_u c^e</i>	
		<i>s_e M_h b_z H</i>	
	P	<i>D m_a c^e</i>	
		<i>D m_a b_z H</i>	1	
		<i>s_e M_h S_b c^e</i>	
		<i>s_e M_h b_z</i>	1	
		<i>h c_u S_b H</i>	
		<i>h c_u</i>	1	
1,2,3		<i>h M_h c_u S_b</i>	1	..	1
1,2,4		<i>s_e D</i>		.	.	.	1	1
1,2,4		<i>h M_h m_a S_b c^e</i>	1	..	1
1,3,4		<i>h D m_a</i>	1	.	1	
1		<i>s_e h c_u b_z c^e</i>	1	.	1	1	..	1
1		<i>s_e M_h D m_a S_b</i>	1	..	1
3		<i>D m_a S_b b_z c^e</i>	.	.		1		1
4		<i>s_e M_h H c^e</i>	1	.	1
1,2	P	<i>s_e D m_a c_u b_z c^e</i>	1	.	1
1,4	P	<i>c^e</i>	1	..	1
		Total	559	76	635	348	47	395

TABLE 2
Offspring from control disloid mothers of the composition indicated; the fathers were $s_a h m_a c_a b_a e'$.

MOTHER	TYPE	0	1	2	3	4	1,2	1,3	1,4	2,3	2,4	3,4	1,2,3	1,2,4	1,3,4	2,3,4	TOTAL
$+ D m_a S_b +$	I; 15 cultures	1654	258	127	132	151	10	14	33	13	21	21	3	2437
$h + c_a b_a e'$	II; 14 cultures	1849	240	77	119	162	6	7	29	6	15	9	..	1	2	..	2522
$s_a M_h + + H$	I; 13 cultures	1525	289	133	132	205	5	29	33	6	14	4	1	2	2378
$h + c_a b_a e'$	II; 12 cultures	1758	267	102	105	212	1	23	29	11	11	6	1	1	..	3	2530
Total	I; 28 cultures	3179	547	260	164	356	15	43	66	19	35	25	3	..	1	2	4815
	II; 26 cultures	3607	507	179	224	374	7	30	58	17	26	15	1	2	2	3	5052

TABLE 3

+ *H* +Offspring of the cross $S_b + M \quad \varphi \times b_x e^a c_a \sigma^7$. $b_x e^a c_a$

	I; 28 CULTURES	II; 27 CULTURES
Triploids	80	75
♀ Intersexes	353	319
♂ Intersexes	84	107
Superfemales	.	..
Supermales	4	8

Diploids

Type	Class	♀	♂	Total	♀	♂	Total
0	<i>H</i>	215	40	649	189	38	574
	$S_b M$	158	28		141	32	
	$b_x e^a c_a$	180	28		152	22	
4	$S_b H$	9	2	69	8	2	58
	<i>M</i>	13	2		11	1	
	$b_x H$	7	.		9	.	
	$e^a c_a$	15	2		10	2	
	$S_b e^a c_a$	15	..		9	2	
	$b_x M$	3	1		4	.	
5	S_b	27	5	173	24	5	151
	$H M$	31	5		16	2	
	$H c_a$	24	7		31	4	
	$b_x e^a$	28	6		21	4	
	$S_b c_a$	27	3		29	3	
	$b_x e^a M$	8	2		11	1	

TABLE 3 (continued)

Diploids			I; 28 CULTURES			II; 27 CULTURES		
Type		Class	♀	♂	Total	♀	♂	Total
4,5	R	+	4	..	17	2	..	13
		$S_b H M$	1	..		1	.	
		$b_x H c_a$		1	..	
		e^a	1	..		1	..	
		$S_b e^a M$.		1	..	
		$b_x c_a$	3	1		1	..	
	P	c_a		1		..	.	
		$S_b H c_a$..	1		1	.	
		$b_x H M$	1	.		1	.	
		b_x	1	..		1	..	
		$S_b e^a$..	.		2	.	
		$e^a M$	2	1		.	1	
4		$H e^a c_a$	2	..	2		.	
4,5	R	$S_b b_x e^a M$	1	..	1
		Total	775	135	910	678	119	797

second are the "progressive" doubles in which all three chromosomes are involved two at a time (for example $c_1a_2b_2$). These are indicated in tables 1 and 3 by "R" and "P" respectively.

It will be seen that there is a certain expected amount of crossing over between the two mutant genes treated as markers of a given point (for example between H and e'). In order to incorporate these crossovers the convention was followed that the first region includes the distance from *sepia* to *dichaete*; all other regions extend from the second gene of a given pair to the second gene of the succeeding pair. It is true that a very small amount of crossing over will remain undetected due to the fact that one of the markers of a given point is the normal allelomorph of one of the mutants, but this is insignificant.

TABLE 4

Offspring from control diploid mothers of the composition indicated; the fathers were $b_2 e' c_a$.

MOTHER	TYPE	0	4	5	4,5	TOTAL
+ H +	I; 13 cultures	1684	270	843	64	2861
$b_2 e' c_a$	II; 12 cultures	1206	180	585	25	1996
+ H +	I; 23 cultures	2455	433	1377	57	4322
$S_b + M$	II; 17 cultures	1640	237	766	23	2666
Total	I; 36 cultures	4139	703	2220	121	7183
	II; 29 cultures	2846	417	1351	48	4662

CROSSES IN WHICH ONE THIRD CHROMOSOME WAS MARKED

When the triploid data presented in the preceding section were analyzed the results were so unexpected that it was thought well to repeat the work with entirely different stocks. Accordingly the following triploid cross and its diploid control cross were used:

$$\begin{array}{l}
 1) \quad \frac{r_u h t_h s_i c_u s_r e' c_a}{+} \times \frac{r_u h t_h s_i c_u s_r e' c_a}{+} \sigma^1.
 \end{array}$$

$$(2) \quad \frac{r_u h t_h s_i c_u s_r e^s c_a}{+} \varphi \times r_u h t_h s_i c_u s_r e^s c_a \sigma.$$

The technique employed was quite similar to that described for the previous crosses except that ten males instead of five were used for every female in order to insure proper fertilization. The symbols represent the following mutant genes which have the loci indicated on the standard diploid map: r_u , roughoid at 0.0; h , hairy at 26.5; t_h , thread at 42.2; s_i , scarlet at 44.0; c_u , curled at 50.0; s_r , stripe at 62.0; e^s , sooty at 70.7; c_a , claret at 100.7.

In utilizing the data of cross (1) a correction must be made for undetected crossing over between the two similar wild-type chromosomes. If we consider any given region, for example region (4) between scarlet and curled, the amount of crossing over between the marked chromosome and the two wild-type chromosomes is known. But the amount of crossing over in region (4) between the two wild-type chromosomes is not known. If we assume this latter to be half the former (and, as will be seen, the results justify this assumption), then the crossing over for all three chromosomes will be $1\frac{1}{2}$ times the detected crossing over. (This correction, it should be noted, has nothing to do with a correction for sister-strand crossing over.)

A more explicit demonstration of this relationship is quite simple, but tedious. A brief resumé follows.

We may indicate the marked chromosome of the triploid by $a_1a_2a_3a_4a_5a_6a_7a_8$; and the wild-type chromosomes of this triploid by $b_1b_2b_3b_4b_5b_6b_7b_8$ and $b_1'b_2'b_3'b_4'b_5'b_6'b_7'b_8'$. We have then individuals of the following composition and appearance which are represented in the proportions indicated. That the various classes of a given type are equal for the first experiment is shown at once by a glance at table 1; it is assumed that the chromosomes of the present experiment behave similarly.

<i>Crossover region</i>	<i>Composition</i>	<i>Appearance</i>	<i>Proportion</i>
0	$a_1a_2a_3a_4a_5a_6a_7a_8$	$a_1a_2a_3a_4a_5a_6a_7a_8$	s
	$b_1b_2b_3b_4b_5b_6b_7b_8$	$b_1b_2b_3b_4b_5b_6b_7b_8$	s
	$b_1'b_2'b_3'b_4'b_5'b_6'b_7'b_8'$	$b_1b_2b_3b_4b_5b_6b_7b_8$	s
			} 3s

	$a_1b_2b_3b_4b_5b_6b_7b_8$	$a_1b_2b_3b_4b_5b_6b_7b_8$	t
	$b_1a_2a_3a_4a_5a_6a_7a_8$	$b_1a_2a_3a_4a_5a_6a_7a_8$	t
	$a_1b_2'b_3'b_4'b_5'b_6'b_7'b_8'$	$a_1b_2b_3b_4b_5b_6b_7b_8$	t
1	$b_1'a_2a_3a_4a_5a_6a_7a_8$	$b_1a_2a_3a_4a_5a_6a_7a_8$	t
	$b_1b_2'b_3'b_4'b_5'b_6'b_7'b_8'$	$b_1b_2b_3b_4b_5b_6b_7b_8$	t
	$b_1'b_2b_3b_4b_5b_6b_7b_8$	$b_1b_2b_3b_4b_5b_6b_7b_8$	t
			6t
2	6 classes	6 classes	6u
3	"	"	6v
4	"	"	6w
5	"	"	6x
6	"	"	6y
7	"	"	6z
1,2	12 classes	12 classes	12c ₁
1,3	"	"	12c ₂
.	.	.	.
.	.	.	.
.	.	.	.
.	.	.	.
5,7	12 classes	12 classes	12c ₂₀
6,7	"	"	12c ₂₁
1,2,3	24 classes	24 classes	24d ₁
1,2,4	"	"	24d ₂
.	.	.	.
.	.	.	.
4,6,7	24 classes	24 classes	24d ₃₄
5,6,7	"	"	24d ₃₅
1,2,3,4	48 classes	48 classes	48e ₁
1,2,3,5	"	"	48e ₂
.	.	.	.
.	.	.	.
3,5,6,7	48 classes	48 classes	48e ₃₄
4,5,6,7	"	"	48e ₃₅

We need not consider the possibility of further multiple crossovers since they do not occur. Their presence would not alter the expressions below except by the addition of terms.

Let us now consider the total amount of crossing over between any two

given loci, for example between loci 4 and 5. By collecting terms we obtain the following expressions:

The total crossing over = $6w + 12(c_3 + c_8 + c_{12} + c_{16} + c_{17} + c_{18}) + 24(d_2 + d_6 + d_{10} + d_{11} + d_{12} + d_{16} + d_{20} + d_{21} + d_{22} + d_{26} + d_{27} + d_{28} + d_{32} + d_{33} + d_{34}) + 48(e_1 + e_5 + e_6 + e_7 + e_{11} + e_{12} + e_{13} + e_{17} + e_{18} + e_{19} + e_{21} + e_{22} + e_{23} + e_{27} + e_{28} + e_{29} + e_{31} + e_{32} + e_{33} + e_{35})$.

The detectable crossing over = $4w + 8(c_3 + c_8 + c_{12} + c_{16} + c_{17} + c_{18}) + 16(d_2 + d_6 + d_{10} + d_{11} + d_{12} + d_{16} + d_{20} + d_{21} + d_{22} + d_{26} + d_{27} + d_{28} + d_{32} + d_{33} + d_{34}) + 32(e_1 + e_5 + e_6 + e_7 + e_{11} + e_{12} + e_{13} + e_{17} + e_{18} + e_{19} + e_{21} + e_{22} + e_{23} + e_{27} + e_{28} + e_{29} + e_{31} + e_{32} + e_{33} + e_{35})$.

That is, the crossing over between loci 4 and 5 is $1\frac{1}{2}$ times the detected crossing over. It may be similarly shown that no matter which two loci are chosen, the crossing over between them is $1\frac{1}{2}$ times the detected crossing over.

Up to this point the discussion has dealt with crossing over and with "crossover values," that is, with the number of crossovers that fall between two given loci for each hundred gametes. But this value cannot be obtained directly, for double crossing over may occur between two given loci without changing the combination of the genes in the loci. By experiments in which other loci between the two given loci are marked, the frequency of these doubles can be determined. These frequencies thus provide corrections by which the percentages of recombination of the genes of the given loci, which is the relation that is directly observed in an experiment, can be transformed into the desired crossover values. For the short intervals dealt with in this paper the amount of this correction is small (see page 241), and the recombination percents are used as indices of the conditions with respect to crossing over.

Table 5 gives the data in complete form for both triploid and diploid crosses in which one chromosome is marked. It should be noticed that the contrary classes of the diploid crosses are on the whole quite well balanced for viability. Indeed, for the type of cross made, the viability relations of the classes involved are unexpectedly good.

The "contrary" classes of the triploid crosses are not, of course, expected to be equal. The wild-type class, for example, includes not only the wild-type non-crossovers, but all the crossovers involving the two wild-type chromosomes alone. But that the viability in the classes of the triploid crosses is good is shown by the strict correspondence between the crossover

values obtained from these triploids and the values obtained from the triploids in which all three chromosomes were marked (see pages 234, 236).

THE RECOMBINATION VALUES

Table 6 presents the recombination values for the first experiment calculated from the data of tables 1, 2, 3, and 4. These figures represent the percentages of *detected* crossed-over chromosomes which emerge. They are not corrected for a possible sister-strand crossing over. The differences between the recombination values for triploid and for diploid, as well as the quotients of these values, are listed separately. It is clear that at the end of the chromosome included in this experiment (the right end) recombination for the triploid is almost half that for the diploid; and that there is a continuous increase in the relative amount of triploid crossing over as we pass from either end of the chromosome to the center. Thus, in the two regions from s_e, h to M_h, D and from S_b, b_x to H, e^* , triploid recombination is only slightly less than diploid recombination. But for the center of the chromosome, triploid recombination is over $3\frac{1}{2}$ times as great as diploid recombination. These relationships hold for both the first and the second sets of cultures. It should be noted that the quotients have relatively low probable errors.

TABLE 5 (continued)

4	$r_u h t_h s_l$	30	4	69		21	4	45	19	10	60		11	7	30
	$c_u s_r e^d c_u$	30	5			19	1		12	19			7	5	
5	$r_u h t_h s_l c_u$	19	1	57		14	1	35	31	33	168		20	15	91
	$s_r e^d c_u$	32	5			19	1		51	53			29	27	
6	$r_u h t_h s_l c_u s_r$	5	..	16		2	1		21	11	116		23	20	111
	$e^d c_u$	10	1			9	2	14	42	42			36	32	
7	$r_u h t_h s_l c_u s_r e^d$	12	1	50		8	..	29	103	85	419		79	65	381
	c_u	33	4			19	2		119	112			123	114	
1,2	$r_u h t_h s_l c_u s_r e^d c_u$	1	..	8		3	..	5	1	..	13		5
	h	4	3			2	..		7	5			3	2	
1,3	$r_u s_l c_u s_r e^d c_u$
	$h t_h$	
1,4	$r_u c_u s_r e^d c_u$	4	..	18		9	3	3	12		4	2	11
	$h t_h s_l$	10	4			8	1		4	2			3	2	
1,5	$r_u s_r e^d c_u$..	1	3		1	..	2	25	19	68		11	8	40
	$h t_h s_l c_u$	1	1			1	.		10	14			13	8	

TABLE 5 (continued)

"Type"	Diploids	I; 20 CULTURES				II; 25 CULTURES				I; 10 CULTURES			II; 10 CULTURES		
		♀	♂	Total		♀	♂	Total		♀	♂	Total	♀	♂	Total
1,6	$r_u e^d c_a$	1	1	5		2	.			16	13	42	14	21	49
	$h t_h s_t c_u s_r$	2	1			2		6	7		9	5	
1,7	$r_u c_a$	3	..	11		2	.			60	44	195	47	42	140
	$h t_h s_t c_u s_r e^d$	7	1			4	..	6		53	38		20	31	
2,3	$r_u h s_t c_u s_r e^d c_a$	1	..	1	
	t_h	
2,4	$r_u h c_u s_r e^d c_a$	1	1	3		3	..	8		2	2	9	2	2	7
	$t_h s_t$	1	..			5	.			4	1		1	2	
2,5	$r_u h s_r e^d c_a$	4		..	.			10	11	44	13	9	33
	$t_h s_t c_u$	4		12	11		7	4	

TABLE 5 (continued)

2,6	$r_u h^e c_u$	1	..	3	5	9	29	6	9	25
	$h_h s_1 c_u s_r$	2		6		4	6	
2,7	$r_u h c_u$	5	..	11	1	.		5	37	140	34	27	113
	$h_h s_1 c_u s_r e^e$	4	2		3	1			30		21	21	
3,4	$r_u h h c_u s_r e^e c_u$	2		3
	s_1	..	2		.	3		
3,5	$r_u h h s_r e^e c_u$
	$s_1 c_u$
3,6	$r_u h h e^e c_u$
	$s_1 c_u s_r$
3,7	$r_u h h c_u$	1	2
	$s_1 c_u s_r e^e$	1		..	2	
4,5	$r_u h h s_1 s_r e^e c_u$	1	..	6	2	..		4	1	5	..	1	1
	c_u	5	..		2	..			2		
4,6	$r_u h h s_1 e^e c_u$	1	..	4	4	11	1	..	1
	$c_u s_r$	2	1		4		

TABLE 5 (continued)

"Type"	Diploids	I; 20 CULTURES				II; 28 CULTURES				I; 10 CULTURES			II; 10 CULTURES		
		♀	♂	Total	♀	♂	Total	♀	♂	Total	♀	♂	Total	♀	Total
4,7	$r_a h t_h s_i c_a$	4	1	14	3	..	6	9	5	29	4	7	27	4	7
	$c_a s_r e'$	8	1		2	1		6	9		9	7			
5,6	$r_a h t_h s_i c_a e' c_a$	2	2
	s_r		2	..		1	1		1	1
5,7	$r_a h t_h s_i c_a c_a$	1	..	6	4	8	4	37	10	5	37	10	5
	$s_r e'$	5	..		4	..		12	13		11	11		11	11
6,7	$r_a h t_h s_i c_a s_r c_a$	2	..	4	3	18	10
	e'	2	..		3	..		11	7		3	7		3	7
1,2,4	$r_a t_h s_i$	2	..	2	1	1	..	1	2	..	1
	$h c_a s_r e' c_a$		1	..		1	..

TABLE 5 (continued)

1,2,5	$r_u t_h s_i c_u$	1	1	2	3	1
	$h s_r e' c_u$	1	
1,2,6	$r_u t_h s_i c_u s_r$.	..	1	2	3
	$h e' c_u$	1				2	1	
1,2,7	$r_u t_h s_i c_u s_r e'$	2	..	2	.		.		9	2	..	8
	$h c_u$					6	
1,4,5	$r_u c_u$				1	1	..	1
	$h t_h s_i s_r e' c_u$	
1,4,6	$r_u c_u s_r$	1	.		1	1	.	1	1
	$h t_h s_i e' c_u$	
1,4,7	$r_u c_u s_r e'$		1	8	1	1	5
	$h t_h s_i c_u$	1	.				2	1	
1,5,6	$r_u s_r$			1	1	..	1
	$h t_h s_i c_u e' c_u$	
1,5,7	$r_u s_r e'$	2	..	3			23	3	4	11
	$h t_h s_i c_u c_u$	1				2	2	

TABLE 5 (continued)

2,6,7	$r_u h c^e$	1	..	1	2	3	6	2	1	3
	$t_h s_t c_u s_r c_a$	1	1
3,4,5	$r_u h t_h c_u$	2
	$s_t s_r c^e c_a$.	2
3,4,7	$r_u h t_h c_u s_r c^e$	1
	$s_t c_a$..	1
4,5,7	$r_u h t_h s_t s_r c^e$.	..	1	3
	$c_a c_a$	1	2	1
4,6,7	$r_u h t_h s_t c^e$	1	..	1	1
	$c_u s_r c_a$	1	..
1,2,4,5	$r_u t_h s_t s_r c^e c_a$	1
	$h c_u$	1
1,2,4,6	$r_u t_h s_t c^e c_a$	2	2
	$h c_u s_r$
1,2,5,7	$r_u t_h s_t c_u c_a$
	$h s_r c^e$	1	1

TABLE 5 (continued)

"Type"	Diploids	I: 20 CULTURES				II: 25 CULTURES				I: 10 CULTURES			II: 10 CULTURES		
		♀	♂	Total	♀	♂	Total	♀	♂	Total	♀	♂	Total	♀	Total
1, 2, 6, 7	$r_u l_h s_i c_u s_r c_a$	2
	$h c^o$	1	1	..	1	
1, 3, 6, 7	$r_u s_i c_u s_r c_a$
	$h l_h c^o$	1	1	
1, 4, 5, 7	$r_u c_u c_a$
	$h l_h s_i s_r c^o$	1	..	1	
1, 4, 6, 7	$r_u c_u s_r c_a$
	$h l_h s_i c^o$	1	1	

These facts are shown graphically by figure 1, which is from the recombination values for the first set of cultures. Ordinates represent the quotients of the recombination values, abscissae, the arrangement of genes along the chromosome as shown by the standard diploid map. The dotted lines give the respective probable errors for each region. This type of graphic treatment of crossing over data was previously used by MULLER

TABLE 6
Recombination values calculated from the data of tables 1, 2, 3, and 4.

CULTURES I				
REGION	TRIPLOID T	DIPLOID D	DIFFERENCE T-D	QUOTIENT T/D
$s_e, h-M_h, D$	12.3 ± 0.88	14.0 ± 0.34	-1.7 ± 0.94	0.88 ± 0.07
$M_h, D-m_a, c_u$	25.0 ± 1.16	6.9 ± 0.25	18.1 ± 1.19	3.62 ± 0.21
m_a, c_u-S_b, b_x	11.8 ± 0.86	7.4 ± 0.25	4.4 ± 0.90	1.59 ± 0.13
S_b, b_x-H, c^e	8.9 ± 0.49	10.9 ± 0.19	-2.0 ± 0.52	0.82 ± 0.05
H, c^e-M, c_a	20.9 ± 0.91	32.6 ± 0.37	-11.7 ± 1.12	0.64 ± 0.03
Total	78.9 ± 1.98	71.8 ± 1.19		
CULTURES II				
$s_e, h-M_h, D$	13.7 ± 1.17	12.0 ± 0.31	1.7 ± 1.12	1.14 ± 0.10
$M_h, D-m_a, c_u$	19.2 ± 1.34	4.7 ± 0.20	14.5 ± 1.36	4.09 ± 0.29
m_a, c_u-S_b, b_x	10.4 ± 1.04	5.8 ± 0.22	4.6 ± 1.06	1.79 ± 0.19
S_b, b_x-H, c^e	8.2 ± 0.54	9.7 ± 0.20	-1.5 ± 0.58	0.85 ± 0.06
H, c^e-M, c_a	20.7 ± 0.97	30.0 ± 0.45	-19.3 ± 1.07	0.69 ± 0.03
Total	72.2 ± 2.34	62.2 ± 0.65	*	

(1925) in a study of the effects of X-rays. Since the quotients for cultures II are practically identical with those for cultures I (as is demonstrated by the table) it is unnecessary to publish graphs for the former.

Table 7 and figure 2 give the corresponding values for the second experiment. It is obvious that the results of the two experiments are in complete agreement. The effects observed are not, then, to be attributed

to crossover modifiers. This is shown also by the fact that standard values are obtained from the diploid controls.

It should be noted in passing that the strict correspondence between the two experiments is of some theoretical importance. In the first experiment all three chromosomes were marked; in the second experiment only one chromosome was marked, the other two being wild type. The fact

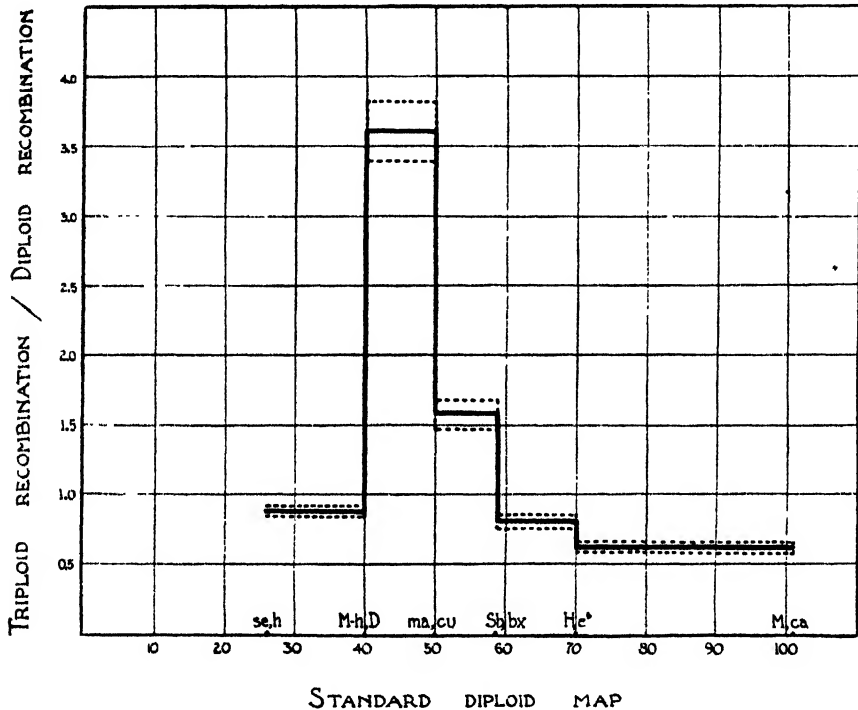


FIGURE 1.—The relation between triploid and diploid recombination along the chromosome. The data are from the first set of cultures of the first experiment. Ordinates represent the ratio of triploid recombination to diploid recombination; abscissae indicate the arrangement of the genes as shown by the standard diploid map. The dotted lines give the probable errors for the respective regions.

that the crossover behavior is the same in the two cases means, therefore, that the chromosomes cross over in the same manner whether they are marked or not. In other words, our assumption of page 220 is justified by the results, and we have definite evidence that crossing over is no more different between wild-type chromosomes than it is between two chromosomes one or both of which are marked. This has long been suspected, but has not previously been demonstrated.

If the assumption is made of free sister-strand interchange and the recombination values are corrected according to the discussion of page 206, we obtain the figures of table 8. A glance at this table will convince one that, although the recombination values are now somewhat different, their quotients still show the same regional differences and still have the same

TABLE 7
Recombination values calculated from the data of table 5.

CULTURES I				
REGION	TRIPLOID T	DIPLOID D	DIFFERENCE T-D	QUOTIENT T/D
r_u-h	19.5 ± 0.90	25.3 ± 0.56	$- 5.8 \pm 1.06$	0.77 ± 0.04
$h-i_h$	14.9 ± 0.81	15.3 ± 0.49	$- 0.4 \pm 0.95$	0.97 ± 0.06
i_h-s_i	1.2 ± 0.25	0.4 ± 0.08	0.8 ± 0.26	3.00 ± 0.87
s_i-c_u	21.2 ± 0.93	5.6 ± 0.30	15.6 ± 0.98	3.79 ± 0.26
c_u-s_r	14.6 ± 0.80	14.0 ± 0.45	0.6 ± 0.92	1.04 ± 0.07
s_r-c^e	6.1 ± 0.54	8.9 ± 0.37	$- 2.8 \pm 0.65$	0.69 ± 0.07
c^e-c_a	18.0 ± 0.87	34.3 ± 0.62	-16.3 ± 1.07	0.52 ± 0.03
Total	95.5 ± 2.02	103.8 ± 1.17		
CULTURES II				
REGION	TRIPLOID T	DIPLOID D	DIFFERENCE T-D	QUOTIENT T/D
r_u-h	18.5 ± 1.10	23.5 ± 0.61	$- 5.0 \pm 1.26$	0.79 ± 0.05
$h-i_h$	13.5 ± 0.96	18.4 ± 0.55	$- 4.9 \pm 1.11$	0.73 ± 0.06
i_h-s_i	1.3 ± 0.32	0.2 ± 0.06	1.1 ± 0.33	6.50 ± 1.60
s_i-c_u	20.6 ± 1.14	4.2 ± 0.29	16.4 ± 1.18	4.90 ± 0.43
c_u-s_r	12.7 ± 0.94	10.4 ± 0.44	2.3 ± 1.04	1.22 ± 0.10
s_r-c^e	5.5 ± 0.65	9.8 ± 0.43	$- 4.3 \pm 0.78$	0.56 ± 0.07
c^e-c_a	15.1 ± 1.01	34.4 ± 0.68	-19.3 ± 1.22	0.44 ± 0.03
Total	87.2 ± 2.42	100.9 ± 1.27		

general order of magnitude. The general conclusions remain unaltered, then, even if sister strands cross over as freely as non-sister strands.

An effect of age on crossing over might be expected to give significant differences between the recombination values for the first set of cultures and for the second set of cultures. Table 9 gives these differences listed separately for diploid and for triploid. The diploid mothers, especially of the first experiment, show a decrease of crossing over with age at central

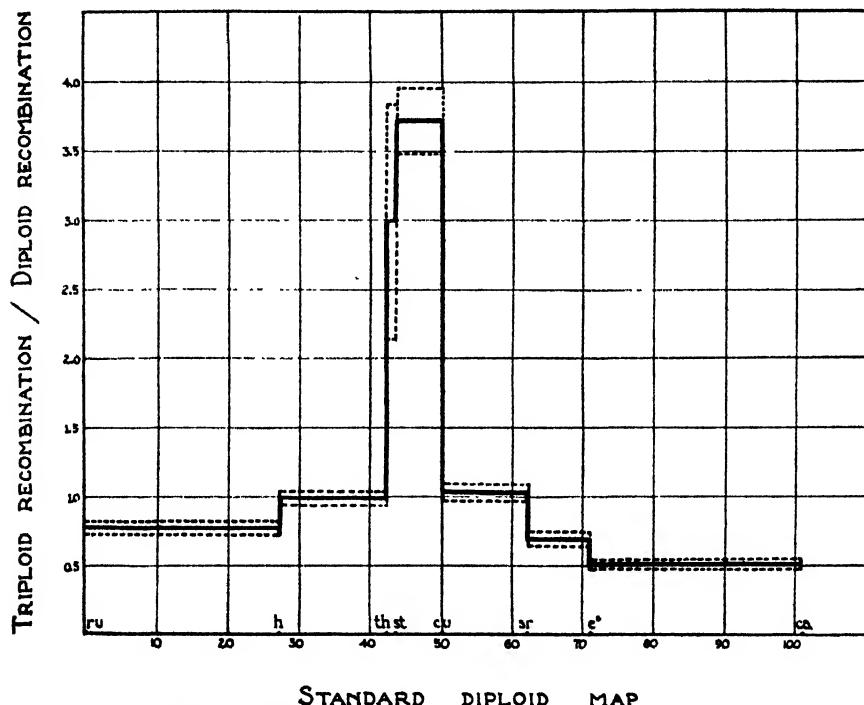


FIGURE 2.—The relations between triploid and diploid recombination along the chromosome. The data are from the first set of cultures of the second experiment. Ordinates and abscissae as in figure 1.

regions. These results are in agreement with those of PLOUGH (1921) and of BRIDGES (1927) for this chromosome. But there is no significant difference shown by the present data between the two sets of triploid cultures. This does not necessarily demonstrate the absence of an effect of age on crossing over in triploids. It is conceivable that such an effect exists, but that it is too delicate to be shown by the data, or that it does not coincide in its time relationships with that shown by diploids. At any rate it is obvious that there are differences between the age change in

diploids and in triploids. It is possible that this fact has some significant relation to the relative lengthening of the triploid chromosome in the center.

TABLE 8
Recombination values corrected on the basis of free sister-strand interchange.

CULTURES I					
REGION	TRIPLOID	DIPLOID	REGION	TRIPLOID	DIPLOID
$s_a, h-M_h, D$	15.4 ± 0.97	21.0 ± 0.40	r_u-h	24.4 ± 0.97	37.9 ± 0.63
$M_h, D-m_a, c_u$	31.2 ± 1.24	10.3 ± 0.30	$h-t_h$	18.6 ± 0.88	22.9 ± 0.55
m_a, c_u-S_b, b_x	14.7 ± 0.95	11.1 ± 0.31	t_h-s_t	1.5 ± 0.28	0.6 ± 0.10
S_b, b_x-H, e^a	11.1 ± 0.54	16.3 ± 0.23	s_t-c_u	26.5 ± 1.01	8.4 ± 0.36
H, e^a-M, c_a	26.1 ± 0.98	48.9 ± 0.40	c_u-s_r	18.2 ± 0.88	21.0 ± 0.53
			s_r-e^a	7.6 ± 0.60	13.3 ± 0.44
			e^a-c_a	22.5 ± 0.95	51.4 ± 0.65
Total	98.5 ± 2.15	107.6 ± 0.75		119.3 ± 2.20	155.5 ± 1.32

CULTURES II					
REGION	TRIPLOID	DIPLOID	REGION	TRIPLOID	DIPLOID
$s_a, h-M_h, D$	17.1 ± 1.28	18.0 ± 0.36	r_u-h	23.1 ± 1.19	35.2 ± 0.68
$M_h, D-m_a, c_u$	24.0 ± 1.45	7.0 ± 0.24	$h-t_h$	16.9 ± 1.06	27.6 ± 0.64
m_a, c_u-S_b, b_x	13.0 ± 1.14	8.7 ± 0.28	t_h-s_t	1.6 ± 0.36	0.3 ± 0.08
S_b, b_x-H, e^a	10.2 ± 0.59	14.5 ± 0.24	s_t-c_u	25.7 ± 1.24	6.3 ± 0.35
H, e^a-M, c_a	25.9 ± 1.05	45.0 ± 0.49	c_u-s_r	15.9 ± 0.92	15.6 ± 0.52
			s_r-e^a	6.9 ± 0.72	14.7 ± 0.51
			e^a-c_a	18.9 ± 1.11	51.6 ± 0.71
Total	90.2 ± 2.55	93.2 ± 0.75		109.0 ± 2.61	151.3 ± 1.43

It is the regional variation of crossing over along the chromosome with which we are primarily concerned. The regions of the third chromosome most affected by triploidy are the same regions most affected by temperature (PLOUGH 1921) and by X-rays (MULLER 1925). If the graphs of

figures 1 and 2 of the present paper are compared with the graphs for chromosome III of MULLER's paper, it will be seen that they are curiously alike. Indeed the only apparent difference is one of degree of effect; triploidy gives more extreme results than does the heavy X-ray dosage of MULLER. These similarities, as we shall see, have no direct significance. The results obtained by the subsection of diploids to temperature and to X-rays are correlated with the distance of the region involved from the spindle-fibre attachment. A comparison of recombination values for

TABLE 9

Age change in crossing over as shown by the recombination for cultures I minus the recombination for cultures II. From tables 6 and 7.

REGION	TRIPLOID	DIPLOID	REGION	TRIPLOID	DIPLOID
s_a-h-M_h,D	-1.4 ± 1.46	2.0 ± 0.46	r_u-h	1.0 ± 1.42	1.8 ± 0.83
$M_h,D-m_a,c_u$	5.8 ± 1.77	2.2 ± 0.32	$h-l_h$	1.4 ± 1.26	-3.1 ± 0.74
$m_a,c_u-S_b^*,b_x$	1.4 ± 1.35	1.6 ± 0.33	l_h-s_t	-0.1 ± 0.41	0.2 ± 0.10
S_b,b_x-H,e^a	0.7 ± 0.73	1.2 ± 0.28	s_t-c_u	0.6 ± 1.47	1.4 ± 0.42
H,e^a-M,c_a	0.2 ± 1.33	2.6 ± 0.58	c_u-s_r	1.9 ± 1.23	3.6 ± 0.63
			s_r-e^a	0.6 ± 0.84	-0.9 ± 0.57
			e^a-c_a	2.9 ± 1.33	-0.1 ± 0.92

chromosomes I, II, and III from such treated diploid females shows, so far as the data go, that the effect is greatest at the region of spindle-fibre attachment and decreases progressively from that region. This, as we have seen, is true also of chromosome III of triploids.

But when we consider chromosome I of triploids, and compare the data with those for chromosome III, we find that the results are exactly opposite in the two chromosomes with respect to distance from the spindle-fibre attachment. BRIDGES and ANDERSON (1925) found the following variation along chromosome I: crossing over in the extreme left end (from yellow, 0.0 to bifid, 6.9) was twice as high as in the diploid controls; but in regions to the right of bifid it was only about one-half as high. The right end of the chromosome is the region of spindle-fibre attachment. In the first chromosome, then, the increase is at the distal end of the chromosome with respect to the spindle-fibre attachment. If the data of BRIDGES and ANDERSON are plotted in the same manner as the data of the present paper

a curve is obtained similar in form to one-half the curve of figure 2. This would seem off-hand to correspond with the fact that the X-chromosome is rod shaped, whereas the third chromosome is V-shaped (that is, consists of two rods fastened together). But the maximum of the curve for chromosome III falls at the region of spindle-fibre attachment, and the maximum of the corresponding curve for chromosome I falls at the opposite end of the chromosome with respect to the attachment.

However, although the differences between triploid and diploid crossing over are not to be explained on the basis of a differential effect depending upon distance from the spindle-fibre attachment, it is a fact that a correlation exists between the lengthening or shortening of the triploid map and the spacing of the genes in the chromosomes as exhibited by the diploid map. Regions in which genes are clumped in the diploid, that is, in which there is relatively little crossing over between successive genes, are longer in the triploid; and, conversely, regions in which genes are far apart in the diploid, that is, in which there is relatively much crossing over between successive genes, are shorter in the triploid. This is true both for chromosome I and chromosome III. It seems reasonable to suppose, therefore, that triploid crossing over gives us a more dependable measure of the actual physical distance between genes than does diploid crossing over.

MULLER, ALTENBURG, and PAINTER (MULLER and ALTENBURG 1928, MULLER and PAINTER 1929) have recently reached conclusions of much interest in this connection from data of an entirely different nature. In a study of translocations produced in the diploid by exposure to X-rays these investigators find that regions in which genes are normally clumped are seen on translocation to be longer than the genetic results had previously indicated, whereas regions in which genes are normally sparse are seen to be shorter. That is, the scale of our diploid maps varies from region to region. If the contention of the present paper is justified, the scale of the triploid maps varies less than the scale of the diploid maps.

It may be stated incidentally that incomplete data upon crossing over in the second chromosome of triploids give results similar in direction but not so extreme in absolute value as those for the third chromosome of triploids.

The cytological studies of triploid *Drosophilas* by METZ (1925) led him to suggest that the peculiar genetic results obtained for the X-chromosome by BRIDGES and ANDERSON were a reflection of the observed anomalous behavior of the X-chromosomes. "The autosomes appear to associate uniformly throughout their length, but the X-chromosomes seem to behave differently. In most figures the latter are closely associated for about

half their length, and then diverge, finger-like as shown by the drawings. This may be due to a precocious separation of the homologues at one end, preceded by a state of close association throughout." The fact that chromosome III exhibits an even greater regional differentiation than the X would indicate that the correlation observed by METZ was of no direct causal significance.

CORRECTION CURVES AND MAPS

Correction curves may be constructed for the triploid data according to the method of BRIDGES (BRIDGES and MORGAN 1923) for the diploid, giving by a process of extrapolation the correction for double crossing over for the region between any two successive genes. The data of the first experiment are hardly amenable to such treatment since two crosses were necessary in covering the chromosome. Correction curves from the trip-

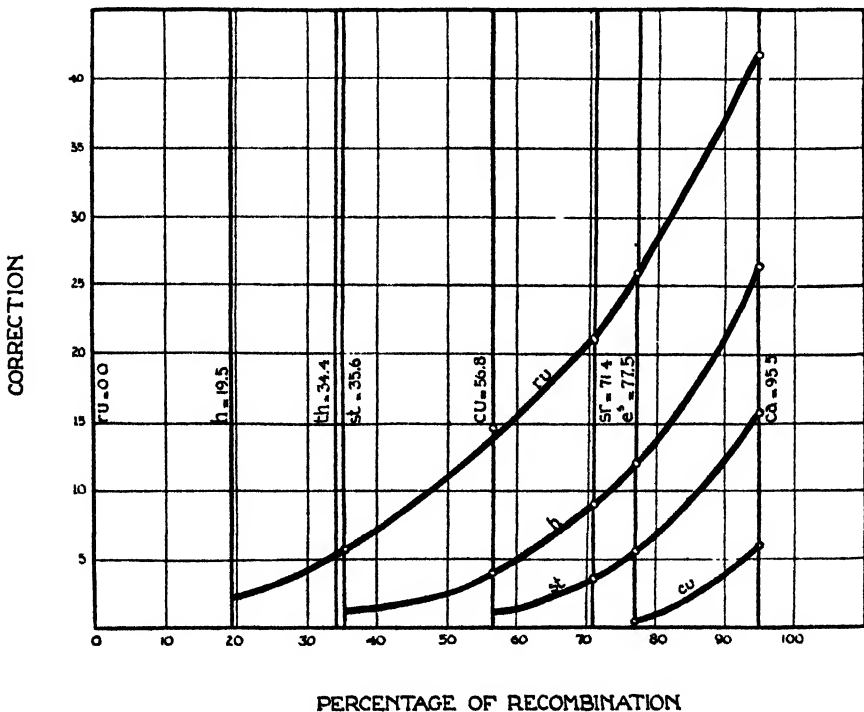


FIGURE 3.—Correction curves to give map distances for the third chromosome of triploids. The data used are from the first cultures of the second experiment. Ordinates represent the correction to be added to the respective recombination percentage to give the crossover value. The difference between the abscissae of two *succeeding* vertical lines represents the recombination value for the corresponding genes.

loid data of the second experiment are given in figure 3. Corresponding correction curves from the diploid data were practically identical with those published by BRIDGES and MORGAN (1923) and are therefore not given here. The ordinates of figure 3 represent the corrections to be added to the respective recombination percentages in order to give the correct crossover values, that is, the map distances. The difference between the abscissae of two succeeding vertical lines gives the recombination between the corresponding genes; this does not apply to genes separated by one or more mutant genes of the cross. The open circles of each curve show for the indicated gene the corrections calculated from the experimental data. These curves may be extended smoothly from the last circle toward the base line giving the approximate correction between successive loci. These corrections make no pretense to a high degree of accuracy. They will, however, facilitate somewhat the prediction of crossover values for chromosome III in triploid *Drosophila*s.

The one suggestive difference between these curves and those for the diploid depends upon the fact that the triploid curves begin with the first open circles at higher levels, at least for the ends of the chromosome. If this difference is significant it would indicate that double crossing over is higher in the triploid than in the diploid at the ends of the chromosome, in spite of the fact that the triploid recombination values are about half the diploid values. In other words, the reduction in crossing over at the ends of the triploid chromosome is dependent upon a considerable reduction in single crossing over, and exists in spite of a possible increase in double crossing over.

For the center of the chromosome there is no detectable difference between the corrections for triploid and for diploid. So far as the data go, therefore, they would indicate that there is a very marked increase in single crossing over in central regions of the triploid chromosome which entirely overshadows an apparent equality in double crossing over. These differences, however, should not be emphasized for it is not certain that they are significant.

Corrected maps for the triploid and diploid may be compared in figure 4. These maps are constructed similarly from the data of the first cultures of the second experiment. The values for the diploid are slightly higher than those of the standard diploid map, but this is to be expected since in the present experiments data from young and old females were segregated, which is not the case in ordinary crossover work. The genes used in the first experiment were omitted since the correction curves for these crosses are less accurate than are those of figure 3.

RECURRENT AND PROGRESSIVE DOUBLE CROSSOVERS

It is of interest to consider the relative occurrence of recurrent and progressive double crossovers. Obviously only the results of the first experiment are of value in this connection. When the proper data are selected

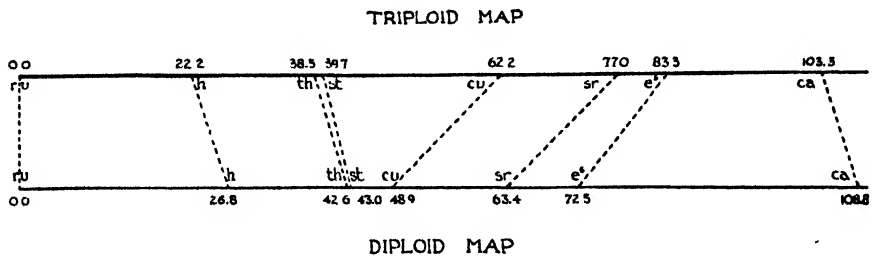


FIGURE 4.—A comparison of triploid and diploid maps for the third chromosome. The data are from the first cultures of the second experiment. Dotted lines connect the same genes.

from tables 1 and 3, table 10 is obtained. This table includes not only the simple double crossovers, but also the four triples of table 1, for a triple may be regarded as a succession of two double crossovers.

TABLE 10

A comparison of recurrent and progressive double crossovers. The data are taken from tables 1 and 3.

	RECURRENT	PROGRESSIVE	DIFFERENCE
Cultures I	43	43	0
Cultures II	20	26	6
Total	63 = 47.7% ± 2.9%	69 = 52.3% ± 2.9%	6 = 4.5% ± 4.1%

It is clear that the recurrent doubles and the progressive doubles are equal in number. It follows therefore that the chromosomes taking part in the first of two crossovers have no influence on the chromosomes taking part in the second. Or more accurately, when we recall that six crossover strands are present instead of three, one of the two strands which have crossed over at a first level crosses over at a second level as freely with a strand from the third chromosome as with a strand from the original two chromosomes. This would indicate that synapsis involves all three chromosomes equally. It would indicate further that the interference effects in triploids are not appreciably different for two successive crossovers in the same two strands than they are for two successive crossovers involving three strands. For if interference in the first case were greater, as one might

possibly be led to expect on a *a priori* grounds, it would follow that progressive doubles would be more frequent than recurrent doubles. The equality of the two types of double crossovers observed here is quite in accord with the similar results of BRIDGES and ANDERSON (1925) for the X chromosome. These investigators record 15 recurrent and 10 progressive doubles. This would make a sum total of 78 recurrences and 79 progressives observed to date.

Coincidence values are not, strictly speaking, comparable in diploid and triploid. For coincidence is defined as the ratio of the actual percentage of doubles in two given regions to the expected percentage of doubles in these regions. Now the percentage of doubles expected as a matter of chance is obtained by multiplying together the crossover percentages of the two regions involved. It is obvious that this product represents the expectation of actual doubles (as exhibited by the emerging chromosomes) only in case two strands are in synapsis. It cannot be the expectation in four-strand crossing over, for the two separate crossings over do not necessarily involve the same single strand as central section. Still less is it the expectation in six-strand crossing over. Of course one may form the product irrespective of its significance and divide the observed percentage of doubles by it, thus obtaining an expression for "coincidence." This expression has no direct meaning in terms of the mechanism involved, but it is of some value in prediction and in comparison. "Coincidence" values are therefore given in table 11. Only data from the first experiment are

TABLE 11

"Coincidence" values for triploid and diploid derived from the data of the first experiment.

REGIONS	CULTURES I		CULTURES II	
	TRIPLOID	DIPLOID	TRIPLOID	DIPLOID
1,2	0.87	0.39	0.77	0.35
1,3	0.87	0.94	0.89	0.93
1,4	0.81	0.99	1.69	1.08
2,3	1.17	1.00	0.76	1.54
2,4	0.80	1.01	0.80	1.36
3,4	1.02	0.77	0.37	0.72
4,5	0.86	0.45	0.94	0.34

included here because the actual number of doubles cannot be directly observed in the second experiment.

It is immediately apparent that the values for the triploid are not uniformly higher than are those for the diploid. In this respect the results for the third chromosome are not in agreement with those of BRIDGES and ANDERSON (1925) for the X chromosome.

THE SPINDLE-FIBRE ATTACHMENT

The attachment of the spindle fibre to the third chromosome is seen in cytological preparations to be near the center of the chromosome at the apex of the V. Moreover the genetic evidence has long indicated that this point is somewhere between scarlet and curled near peach (48.0, standard diploid map). Evidence is now at hand from the recent paper of MULLER and PAINTER (1929), from the unpublished work of DOBZHANSKY, and from the data of the present paper to locate this attachment more accurately.

Certain individuals of the second experiment which were not classified in table 5 according to the characters which they displayed may now be considered. They include the so-called equational exceptions, that is, individuals which have received two third chromosomes from their mothers, these two chromosomes being derived wholly or in part from sister strands. Such exceptions will, of course, be detected only in case they are equational for one of the recessive mutant genes present in the mother in single dose. They will necessarily occur only among the triploid, intersex, and supermale offspring, since these alone have the requisite number of third chromosomes. Unfortunately the characters roughoid, hairy, and thread could not be classified with certainty in these experiments in intersexes; hence they were disregarded in formulating table 12.

If we count the number of times each gene is represented in this table we have the following graded series: s_t , 6; c_w , 6; s_r , 13; e^* , 16; c_a , 26. Now the number of representations of each gene expected as a matter of chance is 73.7. This figure is obtained as follows. If we consider any given locus, for example the scarlet locus, it is obvious that there are present in the six strand stage two scarlet sister genes and four normal allelomorphs. If in the formation of the diploid egg chance alone determines which two of these six allelomorphs pass to the given pole, $2/30$, that is, $1/15$, of the diploid eggs will contain two scarlet genes. Now the total number of eggs diploid for the third chromosome which are to be considered is represented by the sum of the total numbers of triploids, female intersexes, male intersexes, and supermales; this sum is 1106. One fifteenth of this sum is, then, 73.7. The fact

that the representations fall much below expectation would indicate that the distribution is not a chance distribution. Another obvious relationship is that the genes form a graded series according to their distance from the center of the chromosome.

TABLE 12
Equational exceptions for the second experiment.

CLASS	TRIPLOIDS	♀ INTERSEXES	♂ INTERSEXES	SUPERMALES
s_t	1	2	1	1
$s_t c^a$	1
$c_u s_r c^a$	1	.
$c_u s_r c^a c_a$..	2	3	..
s_r	1	..
$s_r c^a$..	1
$s_r c^a c_a$	1	1	3	.
c^a	1	1	.	.
$c^a c_a$..	1
c_a	1	10	5	..

A similar graded series of representations of genes in equational exceptions from triploids was found by BRIDGES and ANDERSON (1925) for the X chromosome. For this chromosome the number of equationals is lowest for the right end and increases progressively toward the left end. It is assumed by these investigators that separation of sister genes always occurs at the spindle fibre. Equational exceptions at loci not at the region of spindle-fibre attachment depend, then, upon crossing over and a resulting association of sister genes which would otherwise have been separated. The amount of crossing over would increase, obviously, with the distance of the locus from the spindle fibre. The attachment of the fibre to the X chromosome is therefore supposedly at the right end. The same conclusion had previously been reached by a similar consideration of the equational exceptions occurring in experiments with attached X chromosomes (ANDERSON 1925, L. V. MORGAN 1925 and unpublished work of STURTEVANT).

According to the same argument the spindle-fibre attachment to the third chromosome could not be to the right of curled. The fact that in no case do scarlet and curled appear in the same equational exception of table 12 would seem to demonstrate that the attachment is between these two genes. It may be stated that for those equational exceptions in which roughoid, hairy, and thread were readily classified, that is, in the triploids and supermales, the number of representations is as follows: r_u , 6; h , 4; l_h , 3; s_i , 3; Although these numbers are low they indicate, as expected, that the spindle fibre is not to the left of scarlet. The present data, therefore, place the attachment at the center of chromosome III between scarlet and curled. This location was previously suggested by the data of BRIDGES from preliminary crosses with triploid females designed to determine the attachment (MORGAN, STURTEVANT, and BRIDGES 1925).

These conclusions regarding spindle-fibre attachment are in complete agreement with those recently reached by MULLER and PAINTER (1929) and by DOBZHANSKY (unpublished) from a study of translocations. MULLER and PAINTER report a translocation in which slightly less than half of the third chromosome has broken away and has become attached to the end of the second chromosome. The cytological preparations demonstrate clearly that this break is just to the left of the spindle-fibre attachment. The genetic position of the break is, moreover, slightly to the left of the center of the linkage map half way between scarlet and pink—that is, at about 46 on the diploid map. This evidence, in conjunction with the evidence from triploids discussed above, would then place the attachment in a region limited by loci 46 and 50 of the standard diploid map.

The work of DOBZHANSKY offers further confirmation. In a study of translocations of pieces of the third chromosome to the fourth chromosome two cases were found in which the break occurred very near the spindle fibre, one just to the left of the fibre, and one to the right. These cases make possible the location of the spindle fibre with respect to the mutant genes marked. For from a study of the crossing over relationships it is clear that scarlet is to the right of the first break, and curled to the left of the second. The appearance of the chromosomes, as well as the crossover relations, gives DOBZHANSKY an opportunity to estimate the distances from these genes to the spindle fibre the attachment of which he accordingly places between loci 46 and 48.

DOBZHANSKY's results are of further interest in connection with crossing over in triploids, for they show that, for central regions of the third chromosome, distances between genes are much longer than our standard diploid maps would indicate. This is obviously in agreement with the similar

results of MULLER and PAINTER (1929) and with the conclusions of the present paper (see page 240).

CELL SIZE AND CROSSING OVER

That there are marked differences between triploid and diploid crossing over cannot be questioned, and it would seem clear that triploid crossing over more nearly represents the actual distance between genes than does diploid crossing over, but the cause of the differences remains quite obscure. So far as we know, the crossing over system in triploid *Drosophila*s differs from that in the diploids in two respects. In the first place there are present six crossing over strands instead of four, and secondly the triploid cell is much larger than the diploid cell. The former follows from the data of BRIDGES and ANDERSON (1925), and the latter has been demonstrated by DOBZHANSKY (1929).

It is conceivable that the presence of six crossing over strands gives rise *per se* in some manner to the differences; they are not, however, as has been shown, the mere direct concomitant of sister-strand crossing over. On the other hand it seems plausible that marked alterations in the size of the cell-components might involve alterations in the relations of chromosomes to cytoplasm, and thus result in crossover variations.

There seem to be two immediate methods of attacking these problems. We may reduce the number of crossover strands in the triploid cell, and we may (possibly?) alter the size of the diploid cell. A reduction of the number of crossover strands is brought about readily by the proper use of crossover reducers. Crosses of this type are now well under way, but are not ready to report.

It was hoped, in the second place, that the diploid cell size of the mutant giant might be comparable to that of the normal triploid cell. The work of GABRITSCHESKY and BRIDGES (1928) had shown that giant and non-giant females from giant stock do not differ genetically and that the crossover values from these two types are normal and equal. It was known further from measurements of the chromosome plates published by these investigators that the chromosomes of giant females do not differ in length, and presumably also not in thickness, from ordinary diploid chromosomes. If giant cells proved to be larger than non-giant cells we should, therefore, have the altered diploid system desired.

Accordingly enough measurements were made of the cell size of giant and of non-giant females from giant-bobbed¹¹-attached-X stock to detect any differences. These measurements were made with the assistance of Mr. IRWIN SPRITZER using the technique of DOBZHANSKY. Bristle counts

were made in three specific different regions of the wing. Since it is known that each cell of the wing is provided with a single bristle, it follows that the cell size is inversely proportional to the number of bristles per area. Table 13 gives these results as well as the results for normal female, normal male, and normal triploid taken from the paper of DOBZHANSKY.

TABLE 13

Number of bristles per 0.01 mm² in three given regions of the wing. The figures for 2N ♀, ♂, and 3N ♀ are from DOBZHANSKY. The giant females and non-giant females were from giant bobbed¹¹-attached-X stock.

	REGION I	REGION II	REGION III
giant ♀	56.02 ± 0.58	63.12 ± 0.61	57.37 ± 0.50
non-giant ♀	59.82 ± 0.66	67.04 ± 0.56	61.38 ± 0.52
difference	3.80 ± 0.88	3.92 ± 0.83	4.01 ± 0.72
2N ♀	50.76 ± 0.22	61.01 ± 0.31	55.67 ± 0.29
♂	57.42 ± 0.35	70.46 ± 0.36	63.02 ± 0.33
3N ♀	36.70 ± 0.22	44.47 ± 0.27	41.35 ± 0.25

These figures demonstrate a difference which is just significant between the cell size of the giant female and that of the non-giant female; the two types are from the same culture and are presumably of identical composition. DR. BRIDGES informs me that preparations of the ovaries of giant females show rare cells diploid in composition and distinctly larger in size than the surrounding cells. It is possible that similar occasional large cells are present in the wing and account for the slightly larger average size of cells of the giant as compared with the non-giant, as shown in the table. The cells of the latter are somewhat different in size from those of the normal diploid female, but this is no doubt to be explained on the basis of the presence of different modifiers in the two stocks.

At any rate it is perfectly clear that the size of the triploid cell is of an entirely different order of magnitude from the size of cells of giant diploid females. It follows, then, that the counts from giant stock have no relation to the work on crossing over in triploids. They have been mentioned here because they have some incidental interest of their own. It would seem that, since the size of giant and of non-giant cells is not very different, the

distinctly greater size of the giant fly is dependent (at least partially) upon a greater number of cells. The rare presence of large cells in the ovaries, however, renders such a conclusion tentative until the matter is investigated further.

CONCLUSIONS

(1) In *Drosophila melanogaster* crossing over in the triploid shows marked regional differences along the third chromosome from crossing over in the diploid. Crossing over at the ends of the chromosome is slightly more than one-half as great in the triploid as in the diploid; and as we pass from either end of the chromosome to the center the relative amount of crossing over in the triploid increases continuously until we reach a maximum at the center, for which region triploid crossing over is more than three times as great as diploid crossing over.

(2) A comparison of these results with those of BRIDGES and ANDERSON for the X chromosome of triploids results in the conclusion that the variations are not correlated with the distance of the region from the spindle-fibre attachment.

(3) They are correlated, however, with the regional spacing of the genes as represented by the diploid maps. Regions in which genes are closely spaced on the diploid maps are lengthened in the triploid; and regions in which genes are far apart on the diploid maps are shortened in the triploid.

(4) It is probable, therefore, that the triploid maps represent more accurately than the diploid maps the actual spacing of the genes along the chromosomes.

(5) The data do not show any effect of age upon crossing over in the third chromosomes of triploids, although the usual effect is shown by the diploid controls.

(6) Crossing over takes place between two wild-type chromosomes to the same extent that it does between two chromosomes one or both of which are marked by mutants.

(7) For chromosome III the number of recurrent double crossovers is equal to the number of progressive double crossovers. It follows that the strands taking part in the first crossing over do not in any way determine which strands take part in the second.

(8) The data of the present paper, in conjunction with those of MULLER and PAINTER and of DOBZHANSKY, place the spindle-fibre attachment of chromosome III in the center of the chromosome at some point between loci 46 and about 48 of the standard diploid map.

(9) In attempts to determine the cause of the differences between diploid and triploid crossing over, measurements were made of the cells of females from giant stock. The average cell size of giant females is slightly greater than that of non-giant females from the same stock; it does not approach, however, the size of cells of triploid females.

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THE INHERITANCE, INTERACTIONS AND LINKAGE RELATIONS OF GENES CAUSING YELLOW SEEDLINGS IN MAIZE*¹

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INTRODUCTION

Two genes affecting the production of yellow pigment in maize already have been reported by LINDSTROM (1925). He designated them as l_1 (luteus) and l_2 . The present paper reports two additional genes which have been called l_3 and l_4 . The inheritance of both of these genes, their interactions with a number of other chlorophyll factors, and the linkage relations of one of them are reasonably well established.

GENES l_1 AND l_2

Gene l_1 was reported first by LINDSTROM in 1917. This gene governs the formation of a distinct yellow pigment but has no effect upon the green pigments. The action of l_1 , therefore, may be observed only in such genotypes as suppress the development of the green pigments either partially or entirely. With the proper genotype the action of l_1 may be observed both in seedlings and mature plants. In the latter, l_1 is best known in the japonica type of striping. With l_1 such japonica plants have alternate stripes of green and yellow, whereas if L_1 is present the stripes are green and white. With the proper genotype, l_1 was found by LINDSTROM to occur as a pure-yellow or a virescent-yellow seedling. He has summarized the interactions of l_1 (LINDSTROM 1925) with the typical seedling genes as follows:

L_1W_1V ,	green
l_1W_1V ,	green
L_1W_1v ,	virescent-white
L_1w_1V ,	white or albino
L_1w_1v ,	white or albino
l_1W_1v ,	virescent-yellow
l_1w_1V ,	pure yellow
l_1w_1v ,	pure yellow

The interaction between l_1 and the albino genes w_2 , and w_3 is similar to the interaction of this gene with w_1 .

Gene l_2 also was reported by LINDSTROM (1925). It differs markedly from l_1 both in its appearance and in its interaction with other known chlorophyll genes. The yellow seedlings are deeper in color than those produced by l_1 . Gene l_2 , unlike l_1 , produces yellow seedlings by itself and is lethal in the homozygous recessive condition. In this respect its action is similar to the various albino genes. LINDSTROM found that whereas l_1 gave a 12:3:1 interaction with W_1w_1 , l_2 exhibited a 9:3:4 relation as follows:

9	L_2W_1	green
3	l_2W_1	yellow
3	L_2w_1	white
1	l_2w_1	white

This 9:3:4 relationship also held for the albino genes W_2 and W_3 .

Linkage studies (LINDSTROM 1925) with l_1 and l_2 have placed them about 35 units apart in the R - G linkage group. The order of the genes on this chromosome as determined by LINDSTROM (1925) is L_1 - R - W_2 - L_2 . L_1 and R are linked very closely if not completely.

GENES l_3 AND l_4

The l_3 and l_4 genes were first observed in 1923 in the seedling progenies from plants self-pollinated for the first time during the previous summer. The yellow seedlings produced by these two genes are indistinguishable from those produced by l_2 . The parent plant from which l_3 was obtained came from the commercial variety Iodent, and l_4 came from Clark Yellow Dent. When first isolated l_3 and l_4 were associated with the chlorophyll defect, *iojap* (i_j), the inheritance of which has been reported (JENKINS 1924). Both of these yellow factors, in their interaction with i_j , produced some white-striped-yellow seedlings. When i_j was reported it was mentioned that in some pedigrees segregations of green, white-striped-green, and yellow-striped-green seedlings were obtained, while in other pedigrees the classes were green, white-striped-green, yellow, and white-striped-yellow seedlings. At that time it was not known whether this was due to two different factors for striping or two different factors for yellow. It is now evident that it was due to different factors for yellow seedlings.

INHERITANCE OF l_3 AND OF l_4

The yellow seedlings produced by both l_3 and l_4 are simple Mendelian recessives to the normal green. Both genes when first isolated appeared to be linked with lethal or semi-lethal factors which caused a deficiency in the yellow seedling class and gave distorted 3:1 ratios. Satisfactory 3:1 ratios were not obtained until these cultures had been outcrossed and the disturbing factors eliminated. Critical evidence that the yellow seedlings produced by both l_3 and l_4 were simple recessives was obtained, however, from the proportion of homozygous (LL) and heterozygous (Ll) green plants in progenies from self-fertilized plants which were heterozygous for yellow seedlings, and from the proportion of homozygous and heterozygous plants in progenies resulting from crosses of plants heterozygous for either

$L_3 l_3$ or $L_4 l_4$ with plants of unrelated stocks. In the first case the proportion of homozygous to heterozygous plants should be 1:2 and in the second case 1:1, if a single Mendelian factor is concerned.

Seedling progenies of the original plant and of 150 other plants heterozygous for $L_3 l_3$ have been grown. The progenies contained 12,415 non-yellow and 3,377 yellow seedlings. This is far removed from a 3:1 ratio, Dev. ÷ P. E. being 15.6. A closer approximation to a 3:1 ratio was obtained from 71 F_2 progenies of two crosses between the stock heterozygous for $L_3 l_3$ and unrelated stocks. In this case totals of 5,056 non-yellow and 1,576 yellow seedlings were obtained. Here the Dev. ÷ P. E. was 3.4, still not a particularly good fit.

More critical evidence that only one factor was concerned in the production of l_3 yellow seedlings was obtained from the proportion of homozygous $L_3 L_3$ and heterozygous $L_3 l_3$ normal green plants in progenies segregating for these yellow seedlings and in the F_1 progenies of the cross $L_3 l_3 \times L_3 L_3$. Among 129 self-pollinated green plants from segregating progenies 48 were homozygous and 81 were heterozygous. In this case Dev. ÷ P. E. was 1.4, indicating a good fit. Among 151 self-pollinated plants from F_1 progenies of the cross $L_3 l_3 \times L_3 L_3$, 80 proved to be homozygous and 71 heterozygous. The Dev. ÷ P. E. was 1.1 in this case. From these data it seems safe to conclude that the l_3 yellow seedlings are the result of the action of a single factor.

Progenies have been grown from the original plant and from 75 additional plants heterozygous for $L_4 l_4$. These progenies contained a total of 7,672 non-yellow and 2,045 yellow seedlings. Here, again, there is a large deficiency in the yellow seedling class and very poor agreement with the expected 3:1 ratio. The Dev. ÷ P. E. in this case was 13.3. Much better ratios were obtained among the F_2 and F_3 progenies of a cross between the l_4 stock and an unrelated stock. Progenies from 35 heterozygous F_2 and F_3 plants produced 3,925 non-yellow and 1,231 yellow seedlings. In this case Dev. ÷ P. E. was 2.8, indicating fair agreement between the observed and expected numbers.

Fifty-eight normal green plants in progenies segregating for l_4 yellow seedlings were self-pollinated. The progenies from these plants indicated that 25 of them were homozygous $L_4 L_4$ and 33 were heterozygous $L_4 l_4$. The Dev. ÷ P. E. for the expected 1:2 ratio was 2.4. Data on the progenies from 36 self-pollinated F_1 plants of the cross $L_4 l_4 \times L_4 L_4$ indicated that 18 were homozygous and 18 were heterozygous. These are exactly the expected numbers on the basis of a 1:1 ratio. It may be concluded, therefore,

that the l_4 yellow seedlings are the result of the action of a single genetic factor.

PROOF THAT l_1 , l_2 l_3 AND l_4 ARE DIFFERENT GENES

LINDSTROM (1925) has shown that l_1 and l_2 are different. Crosses between l_1 and l_3 has demonstrated that these genes are different. The F_1 plants of such a cross are normal green, and under the proper conditions dihybrid ratios of green to yellow seedlings are obtained in F_2 . No crosses have been obtained between l_1 and l_4 . However, the phenotypic differences between the yellow seedlings produced by these two genes, the different interactions of the responsible genes with other chlorophyll genes, and the fact that they have different linkage relations leaves little doubt but that they differ genetically.

Genes l_2 , l_3 and l_4 are phenotypically alike, and similar in their interactions with other chlorophyll genes with which they have been tested. All possible combinations have been made between plants heterozygous for these genes. The F_1 plants in each case have been normal green and 9:7 ratios of green to yellow seedlings have been obtained in the F_2 generation. In the case of the cross $L_2l_2 \times L_4l_4$ the 9:7 ratios were modified by linkage as these genes are located on the same chromosome.

INTERACTION OF THE FOUR GENES PRODUCING YELLOW SEEDLINGS WITH CERTAIN OTHER GENES FOR CHLOROPHYLL DEFICIENCY

Dihybrid ratios involving l_1 and i_j

Gene l_1 interacts with i_j in a manner similar to its interaction with j as described by LINDSTROM (1918). The iojap character may be observed in the seedlings and for this reason is a better character to work with than japonica. Self-pollinated plants heterozygous for $L_1l_1 I_ji_j$, give the following dihybrid ratio in F_2 :

$$\begin{array}{rcl} 9 L_1 I_j & \left. \vphantom{\begin{array}{l} 9 L_1 I_j \\ 3 l_1 I_j \end{array}} \right\} & \text{green plants} \\ 3 l_1 I_j & & \\ 3 L_1 i_j & & \text{white-iojap plants} \\ 1 l_1 i_j & & \text{yellow-iojap plants} \end{array}$$

The white-iojap plants are so named because they have alternate stripes of green and of white tissue and the yellow-iojap plants because they have alternate stripes of green and of yellow tissue.

A summary for these two factors is given below:

	$L_1 I_j$ and $l_1 i_j$	$L_1 i_j$	$l_1 i_j$
Observed	5969	1420	503
Expected	5919	1480	493
Deviations	+50	-60	+10
$\chi^2 = 3.06$		$P = 0.22$	

Progenies have been grown from 33 plants heterozygous for both of these factors which were backcrossed to the double recessive ($l_1 i_j$). Ratios of 2 green:1 white-iojap:1 yellow-iojap seedlings were expected. The data from these progenies are summarized below:

	$L_1 I_j$ and $l_1 i_j$	$L_1 i_j$	$l_1 i_j$
Observed	3371	1545	1528
Expected	3222	1611	1611
Deviations	+149	-66	-83
$\chi^2 = 13.87$		$P = 0.001$	

The poor fit in this case is due to a shortage of iojap plants, and in no way indicates linkage as there is very close to a 1:1 ratio of L_1 and l_1 plants among those recessive for i_j .

Dihybrid ratios involving l_2 , l_3 or l_4 and i_j

When plants of the genetic composition $L_3 l_3 I_j i_j$ were self-pollinated and their progenies grown, they contained 4 classes of seedlings in the proportions expected on the basis of independent inheritance. The phenotypic and genotypic description of the seedling classes obtained is given below.

- 9 $L_3 I_j$ green
- 3 $L_3 i_j$ white-iojap
- 3 $l_3 I_j$ yellow
- 1 $l_3 i_j$ white-striped-yellow

The double recessive class is unusual and is very distinct in progenies giving a good clear-cut segregation for iojap.

A summary of the data on 31 progenies from plants heterozygous for these two factors is given below.

	Ll_i	Ll_j	ll_i	ll_j
Observed	2309	725	516	136
Expected 9:3:3:1	2073	691	691	230
Deviations	+236	+34	-175	-94
$\chi^2 = 116.4$		P = very small		

Expected (3:1 segregation
for i_j in the L_3 and l_3
classes)

classes)	2276	758	489	163
Deviations	+33	-33	+27	-27
$\chi^2=7.88$			$P=0.02$	

The deviations from the numbers expected on the basis of a 9:3:3:1 ratio are large. They are due chiefly to the deficiencies in both the l_3 classes however. On the basis of the independent inheritance of these two factors there should be a 3:1 segregation for non-iojap and iojap plants in the L_3 and in the l_3 classes. The deviations computed on this basis are much smaller, P having a value of 0.02. The poor fit is due, however, to deficiencies in the recessive classes and not to linkage.

Data not included in this report show that l_2 and l_4 give interactions with i_j similar to those of l_3 .

Dihybrid ratios involving l_2 , l_3 or l_4 and w_2

LINDSTROM (1925) has shown that plants heterozygous for l_2 and w_1 , w_2 or w_3 give ratios of 9 green, 3 yellow and 4 white seedlings in F_2 when self-pollinated. The segregations of L_2l_2 and W_2w_2 are modified by the linkage between these two factors. Genes l_3 and l_4 have been crossed with w_2 and give similar interactions. In the case of the cross $L_4l_4 \times W_2w_2$ the 9:3:4 ratio also is modified by linkage.

Trihybrid ratios involving l_1 , i_j and l_2 , l_3 or l_4

When plants of the genetic composition $L_1l_1L_2l_2I_1i_1$ were self-pollinated and their progenies grown the following classes of seedlings resulted.

27	$L_1L_1I_1$	} green
9	$l_1l_1I_1$	
9	$L_1L_1i_j$	} white-iojap
9	$L_1l_1i_j$	
3	$l_1l_1I_1$	} yellow
3	$l_1l_1i_j$	
3	$l_1L_2i_j$	} yellow-iojap
3	$l_1l_2i_j$	
3	$L_1l_1i_j$	} white-striped-yellow
1	$l_1l_1i_j$	
		} pale-yellow-striped-yellow

The triple recessive class is difficult to distinguish from the pure-yellow seedlings but has been observed. In these seedlings the deep yellow due to l_3 is suppressed by the i_j factor which in turn allows the lighter l_1 yellow to develop.

Data on the progenies from 89 self-pollinated plants heterozygous for these three factors are presented in table 1.

The data in table 1 do not show a very close fit to the expected numbers. When all of the yellow seedling classes are grouped together the fit is much better but still is not very good. The poor fit, however, is due chiefly to the deficiency of l_3 yellow seedlings.

TABLE 1
Seedling progenies from self-pollinated plants of the composition $L_1l_1L_2l_2I_1i_1$.

PEDIGREE	NUMBER OF PROGENIES	SEEDLING CLASSES					
		GREEN	WHITE-IOJAP	YELLOW-IOJAP	YELLOW	WHITE STRIPED-YELLOW	PALE-YELLOW-STRIPED-YELLOW
1811 ¹	1	117	34	14	55	7	0
1812 ¹	1	104	21	7	44	9	0
2975 ¹	40	2051	558	138	750	71	5
2976 ¹	29	1541	356	115	552	75	8
2980 ²	11	667	178	47	208	31	1
2981 ²	7	357	105	39	115	12	0
Totals	89	4837	1252	360	1724	205	14
<i>Expected</i> (36:9:3:12:3:1)		4721	1180	393	1574	393	131
Deviations		+116	+72	-33	+150	-188	-117
		$\chi^2=218.7$		P=very small			
Rearranged Totals		4837	1252	360	1943		
<i>Expected</i> (36:9:3:16)		4721	1180	393	2098		
Deviations		+116	+72	-33	-155		
		$\chi^2=21.47$		P=0.0001			
Totals for F ₂ progenies							
71		3813	969	274	1401	162	13
Rearranged totals		3813	969	274	1576		
<i>Expected</i> (36:9:3:16)		3731	933	311	1658		
Deviations		+82	+36	-37	-82		
		$\chi^2=11.65$		P=0.009			

¹ F₂ progenies from plants of the composition $L_1i_1l_2l_1I_1L_4$.

² F₂ progenies.

Data not included indicate that the progenies from plants heterozygous for $L_1l_1L_2l_2 I_1i_1$ show similar interactions of the three factors but the ratios are modified by the linkage between l_1 and l_2 . Progenies segregating for $L_1l_1L_2l_2 I_1i_1$ have not been obtained but the indications are that such progenies would give similar interactions but that the ratios would be modified by the linkage between l_1 and l_4 .

Trihybrid ratios involving l_2l_3 , l_2l_4 or l_3l_4 and i_1

When plants of the composition $L_2l_2L_3l_3 I_1i_1$ were self-pollinated their progenies gave the following genotypic and phenotypic classes of seedlings

27 $L_2L_3 I_i$	}	green
9 $L_2L_3 i_i$		white-iojap
9 $L_2l_3 I_i$	}	yellow
9 $l_2L_3 I_i$		
3 $l_2l_3 I_i$		
3 $l_2L_3 i_i$	}	white-striped-yellow
3 $L_2l_3 i_i$		
1 $l_2l_3 i_i$		

Data on the seedling progenies from 4 such self-pollinated plants are recorded in table 2. Because of the usual difficulty in classifying the white-striped-yellow seedlings, both of the yellow classes have been combined, thus making a 27:9:28 ratio of green:white-ijap:yellow seedlings. Excellent agreement between the observed numbers and those expected on the basis of independent inheritance was obtained, P having a value of 0.36.

TABLE 2
Seedlings progenies from self-pollinated plants of the composition $L_2l_3L_3I_i i_i$.

FEDIGREE	NUMBER OF PROGENIES	GREENS	WHITE IOJAPS	YELLOWS	WHITE-STRIPED YELLOWS
4603	4	355	108	285	47
Rearranged totals		355	108	332	
Expected (27:9:28)		335	112	348	
Deviations		+20	-4	-16	

$$\chi^2 = 2.07$$

$$P = 0.36$$

Other data show that similar ratios are obtained when plants of the composition $L_3l_3L_4l_4 I_i i_i$ are self-pollinated. When plants of the composition $L_2l_2L_4l_4 I_i i_i$ are self-pollinated the ratios are modified by the linkage between l_2 and l_4 .

LINKAGE RELATIONS OF l_3

Linkage tests have been made between l_3 and factors in the $R_a-G_{11}, Y-P_1, B-L_9$ and $R-G$ linkage groups. The data so far obtained indicate that l_3 is not located in any of these groups.

Tests of l_3 and i_i

Data already have been presented on l_3 and i_i in connection with the dihybrid $L_3l_3 I_i i_i$ and the trihybrids $L_1l_1L_3l_3 I_i i_i$ and $L_2l_2L_3l_3 I_i i_i$. There was poor agreement between the numbers observed and those expected on the basis of independent inheritance for the first two of these segregations.

For the third segregation, however, P had a value of 0.37 indicating the independent inheritance of l_2 , l_3 and i_j .

The ratios for the dihybrid segregation of $L_3l_3 I_ji_j$ were distorted by deficiencies in the recessive classes. Crossover percentages computed from such distorted ratios would be of doubtful value and so are not included. The trihybrid $L_1l_1L_3l_3 I_ji_j$, however, probably is worthy of more critical examination. The crossover percentages between l_3 and i_j have been computed for this material.

In the progenies from the trihybrid $L_1l_1L_3l_3 I_ji_j$ there appeared to be no linkage between l_3 and i_j . Seventy-one F_2 progenies were used for studying any possible linkage between these two factors. Disregarding the segregation for l_1 the following numbers of seedlings were observed and expected:

	OBSERVED	Expected
green	3813	3730
iojap	1243	1244
yellow	1401	1244
striped-yellow	175	414

YULE's coefficient of association and OWEN's (1928) tables showed 37 percent crossing over in the repulsion phase. This situation does not agree with the manner in which the cross was made. There is an excess in the yellow seedling class and a large deficiency in the striped-yellow seedling class. This is due in part to the difficulty of distinguishing the pure-yellow seedlings and the yellow-striped-yellow seedlings. If these two classes are combined a 9:3:4 ratio results. The expected and observed numbers on the basis of this ratio are:

	OBSERVED	Expected
green seedlings	3813	3730
iojap seedlings	1243	1244
yellow seedlings	1576	1658

For this distribution χ^2 is 5.90 and P is 0.05. This is not a very close fit but there is no evidence of linkage. The crossover percentage as determined by a modification of EMERSON's (1916) formula suggested by COLLINS (1924) was 49.

Probably the most critical data indicating the independent inheritance of l_3 and i_j come from a study of observed and expected numbers of the different kinds of F_3 progeny segregations from the self-fertilized normal green plants in some of the F_2 progenies mentioned above. The observed and expected numbers of F_3 seedling segregations from such plants, together with the corresponding genotype of the parental F_2 plants are recorded below.

Genotypes of the normal green F ₂ plants	F ₂ seedling segregation	Observed	Expected
1 $L_3L_3 I_1I_1$	all green	9	11
2 $L_3l_3 I_1I_1$	3 green:1 yellow	26	21
2 $L_3L_3 I_1i_1$	3 green:1 iojap	25	21
4 $L_3l_3 I_1i_1$	9 green:3 iojap:3 yellow: 1 striped-yellow	35	42

χ^2 for the above distribution is 3.48 and P is 0.34, showing very good agreement between the segregations observed and those expected on the basis of independent inheritance. From this evidence and that previously presented it seems safe to conclude that l_3 and i_1 do not belong in the same linkage group. Unpublished data from recent investigations indicate that i_1 is linked with ramosa and glossy 1. This would preclude l_3 from the R_4-G_H linkage group.

Tests of l_3 with y

Evidence from a study of seedling ratios and the proportions of various kinds of F₂ segregating progenies shows that l_3 and y , a factor for endosperm color, are independently inherited. No F₂ ratios are available but a summary of 11 F₂ seedling progenies is given in table 3.

TABLE 3
F₂ seedling progenies from self-pollinated plants of the composition $L_3l_3 Yy$.

PEDIGREE	NUMBER OF PROGENIES	YELLOW ENDOSPERM		WHITE ENDOSPERM	
		NON-YELLOW SEEDLINGS	YELLOW SEEDLINGS	NON-YELLOW SEEDLINGS	YELLOW SEEDLINGS
2981	11	855	282	304	107
Expected 9:3:3:1		871	290	290	97
Deviations		-16	-8	+14	+10

$$\chi^2 = 2.22$$

$$P = 0.53$$

The segregation agrees excellently with the expected 9:3:3:1 ratio, χ^2 being 2.22 and P being 0.53. It is realized that a summation of several F₂ progenies would obscure a loose linkage as some of the progenies might exhibit coupling and others repulsion and when summed the two conditions would tend to balance each other. A critical examination of the individual progenies to determine whether any of them exhibited either the coupling or repulsion phases of linkage between l_3 and y revealed several progenies in which coupling or repulsion might have existed. These progenies, however, deviate considerably from the expected 3 to 1 ratios for yellow and

white endosperm, and for green and yellow seedlings, preventing an accurate determination of linkage and suggesting that causes other than linkage were operating to distort the ratios for these particular characters.

Additional evidence from a study of the respective numbers of the several kinds of F_3 progenies from self-fertilized green F_2 plants offered further verification that l_3 and y were not linked. The observed and expected numbers of F_3 endosperm and seedling segregations from such plants, together with the corresponding genotypes of the parental F_2 plants are recorded below.

Genotypes of the Green F_2 plants	F_2 endosperm and seedling segregations	Observed	Expected
1 L_3L_3YY	All green from yellow seeds	4	3.1
2 L_3L_3Yy	Green seedlings, 3 yellow:1 white seed	7	6.2
2 L_3l_3YY	Yellow seeds, 3 green:1 yellow seedling	4	6.2
4 L_3l_3Yy	Segregating for both endo- sperm and seedling colors	16	12.4
1 L_3L_3yy	All green from white seeds	2	3.1
2 L_3l_3yy	3 green:1 yellow from white seeds	4	6.2

χ^2 for this distribution is 3.36 and P is 0.65, indicative of excellent agreement between the numbers obtained and those expected on the basis of the independent inheritance of l_3 and y . Gene l_3 , therefore, probably is not located in the $Y-P_1$ linkage group.

Tests of l_3 with l_y

The linkage relations of l_3 and l_y , a simple recessive factor for liguleless leaves, was studied in the F_2 progenies from the cross $L_3l_3l_yl_y \times L_3l_3L_yL_y$. Seedling progenies were grown from 13 self-pollinated plants heterozygous for these two factors. The data on these progenies are summarized in table 4.

Some difficulty was experienced in classifying the yellow seedlings for the liguleless character. Ordinarily these seedlings were smaller than the non-yellow seedlings in the same progenies and the ligules were not well developed. For this reason the two groups of yellow seedlings have been combined, resulting in a 9:3:4 ratio.

Data on the F_2 progenies indicated that l_3 and l_y were not linked and as none of the F_3 progenies seemed to indicate linkage between these two factors the data from the F_2 and the F_3 progenies have been combined. For the F_2 progenies P was 0.13 and for all progenies combined P was 0.49.

Additional evidence that l_3 and l_9 were not in the same linkage group came from a comparison of the actual numbers of the various kinds of F_2 progenies with the numbers expected on the basis of independent inheri-

TABLE 4
Seedling progenies from self-pollinated plants of the composition $L_3l_3L_9l_9$.

PEDIGREE	NUMBER OF PROGENIES	NON-YELLOW SEEDLINGS		YELLOW SEEDLINGS	
		NORMAL	LIGULELESS	NORMAL	LIGULELESS
1812 ¹	1	106	26	36	17
2976 ¹	12	573	233	161	114
2981 ²	8	630	207	194	74
Totals (F ₂ Progenies)		679	259	197	131
Totals rearranged		679	259	328	
Expected (9:3:4)		712	237	316	
Deviations		-33	+22	+12	
$\chi^2=4.03$		P=0.13			
Totals (all progenies) 21		1309	466	391	205
Totals rearranged		1309	466	596	
Expected (9:3:4)		1334	445	593	
Deviations		-25	+21	+3	
$\chi^2=1.48$		P=0.49			

¹ F_2 progenies from plants of the composition $l_3L_9L_9l_9$.

² F_2 progenies.

tance. The self-pollinated green F_2 phenotypes should segregate in the F_3 as shown:

Genotypes of the Green F_2 plants	F_3 seedling segregations	Observed	Expected
1 $l_3L_3L_9L_9$	All green	2	3.1
2 $L_3l_3L_9l_9$	3 green:1 liguleless green	8	6.2
2 $L_3l_3L_9L_9$	3 green:1 yellow	8	6.2
4 $L_3l_3L_9l_9$	9 green:3 liguleless green:3 yellow:1 liguleless yellow	9	12.4
1 $L_3L_3l_9l_9$	All liguleless green	4	3.1
2 $L_3l_3l_9l_9$	3 liguleless green:1 liguleless yellow	6	6.2

The value of χ^2 for the distribution above was only 2.51 and P was 0.77. This is an exceptionally close fit and indicates that l_3 does not belong in the $B-L_9$ linkage group.

Tests of l_3 with genes in the R-G linkage group

Gene l_3 also has been tested for linkage with the following 4 factors

located in the *R-G* linkage group, l_1 , l_2 , l_4 , and R^o . The tests with each of these factors have indicated that l_3 is not located on this chromosome.

l_3 and l_1

Possible linkage relations between l_3 and l_1 may be studied in the F_2 progenies from the trihybrid $L_1l_1L_3l_3I_1i_1$ presented in table 1 and already discussed. The observed numbers do not fit closely those expected on the basis of independent inheritance but the poor fit appears to be due to causes other than linkage between these two factors.

l_3 and l_2

A summary of the data on 6 F_2 progenies from plants of the genetic composition $l_2L_3L_2l_3$ is given below:

	Observed	Expected	Deviation
Non-yellow seedlings	724	687	+37
Yellow seedlings	498	535	-37

The deviation from the 9:7 ratio expected on the basis of independent inheritance is 3.1 times its probable error. The deviation, however, is in the wrong direction to indicate linkage. There is a large deficiency of yellow seedlings whereas there should have been an excess of yellow seedlings if these two factors were linked as they came into the cross from opposite parents.

l_3 and l_4

Gene l_4 is located in the *R-G* linkage group as will be shown by data to be presented later. Data are available on 9 progenies of F_1 plants of the composition $L_3l_4l_3L_4$. The data on these progenies are summarized below:

	Observed	Expected	Deviation
Green seedlings	921	899	+22
Yellow seedlings	677	699	-22

There is fairly close agreement between the observed and the expected numbers. Dev. \div P. E. is 1.7. Here, again, the deviations are in the wrong direction to indicate linkage.

l_3 and R^o

Genes l_1 and R^o are linked very closely, if not completely. As l_3 appeared to be independent of l_1 it naturally would be expected to be independent of R^o . Such proved to be the case. The data on l_3 and R^o come from 15 F_2

progenies from plants of the composition $L_1R'l_2r'$. A summary of the data on these progenies is given below.

	Observed	Expected	Deviation
L_1r' —Non-yellow seedlings with red stems	757	774	-17
L_2R' —Non-yellow seedlings with green stems	276	258	+18
l_2r' —Yellow seedlings with red stems	221	258	-37
l_2R' —Yellow seedlings with non-red stems	122	86	+36

The excess of yellow seedlings with non-red stems is due to the difficulty of distinguishing red stem color on the small yellow seedlings. If all of the yellow seedlings are grouped together much better agreement is obtained between the expected and observed numbers. The results with this grouping, which gives a 9:3:4 ratio, are as follows:

	Observed	Expected	Deviation
Non-yellow seedlings with red stems	757	774	-17
Non-yellow seedlings with green stems	276	258	+18
All classes of yellow seedlings	343	344	-1

P is 0.46 for the above distribution, indicating very close agreement between the numbers observed and those expected on the basis of independent inheritance.

Tetrahybrid $L_1l_1L_3l_3I_i;L_0l_0$

In the linkage studies with l_3 a number of interesting trihybrid and tetrahybrid segregations were obtained and it was thought worth while to report one of them. When plants heterozygous for $L_1l_1L_3l_3I_i;L_0l_0$ are self-pollinated the following genotypic and phenotypic classes of seedlings should result:

81	$L_1L_1I_iL_0$	green
27	$l_1L_1I_iL_0$	green
27	$L_1L_1l_3I_i$	liguleless-green
27	$L_1l_3I_iL_0$	yellow
27	$L_1L_3i;L_0$	white-iojap
9	$l_1l_3I_iL_0$	yellow
9	$l_1L_3i;L_0$	yellow-iojap
9	$l_1L_1I_il_3$	liguleless-green
9	$L_1l_3i;L_0$	white-striped-yellow
9	$L_1l_3I_il_3$	liguleless-yellow
9	$L_1L_3i;l_3$	liguleless-white-iojap
3	$l_1l_3i;l_3$	pale-yellow-striped-yellow
3	$l_1l_3I_il_3$	liguleless-yellow
3	$L_1l_3i;l_3$	liguleless-white-striped-yellow
3	$l_1L_3i;l_3$	liguleless-yellow-iojap
1	$l_1l_3i;l_3$	liguleless-pale-yellow-striped-yellow

Assuming independent inheritance of these four factors, the various phenotypic seedling classes and the observed and expected numbers from four progenies are tabulated below:

Ratio	Phenotypes	Observed	Expected
108	green	137	151
36	liguleless-green	60	50
36	yellow	44	50
27	white-iojap	29	39
12	liguleless-yellow	46	17
9	liguleless-white-iojap	9	13
9	yellow-iojap	14	13
9	white-striped-yellow	7	13
3	liguleless-yellow-iojap	7	4
3	liguleless-white-striped-yellow	1	4
3	pale-yellow-striped-yellow	3	4
1	liguleless-pale-yellow-striped-yellow	2	1

The above distribution even though in fairly good agreement with the expected does not lend itself to statistical analysis because of certain deviations in the yellow-seedling classes which are relatively large, owing, perhaps, to difficulties in correctly classifying striped-yellow and liguleless-yellow seedlings.

If all of the yellow seedling types, exclusive of yellow-iojap, are combined, much better agreement between the actual and calculated numbers is obtained. These results are shown below:

Ratio	Phenotypes	Observed	Expected	Deviation
108	green	137	151	-14
36	liguleless-green	60	50	+10
27	white-iojap	29	39	-10
9	liguleless-white-iojap	9	13	-4
9	yellow-iojap	14	13	+1
3	liguleless-yellow-iojap	7	4	+3
64	yellow seedlings of all classes	103	90	+13

The size of χ^2 for the above distribution was 11.30 and the value of P , 0.08. This fit, although not extremely good, tends to verify the hypothesis of independent inheritance of the four factors, $l_1 l_3 i_1 j_0$.

LINKAGE RELATIONS OF l_4

Linkage tests with l_4 have shown that this factor is located in the R - G linkage group. Linkage data have been obtained with three factor pairs of this group, namely Rr , $L_2 l_2$ and $W_2 w_2$.

Tests of l_4 with R^o

Determinations of the linkage between l_4 and R^o come from data on a number of F_2 and F_3 seedling progenies from plants heterozygous for these two factors. The factor R^o is expressed in the seedlings as a green or at least a non-red stem, whereas seedlings carrying the factor r^r have red stems (provided, of course, that A is present).

Data on 6 progenies showing the repulsion phase of the linkage and 4 progenies showing the coupling phase of the linkage are recorded in table 5.

TABLE 5
Data on the progenies from self-pollinated plants of the composition L_4R^o .

PEDIGREE	RED STEMS		NON-RED STEMS	
	GREEN SEEDLINGS	YELLOW SEEDLINGS	GREEN SEEDLINGS	YELLOW SEEDLINGS
		<i>Repulsion</i>		
4606- 7	66	20	38	3
-11	152	65	62	10
5773- 4	114	36	43	5
5775-17	67	24	31	1
5776-14	103	50	36	11
-16	110	44	37	9
Totals	612	239	247	39
Expected, 37 percent crossing over	607	245	245	39
Deviation	+5	-6	+2	0
	$\chi^2=0.20$		P = very good fit	
		<i>Coupling</i>		
5773-34	46	13	7	3
-41	37	12	7	3
5775-39	209	78	66	35
5776-37	179	11	21	27
Totals	471	114	101	68
Expected, 36 percent crossing over	454	111	111	77
Deviation	+17	+3	-10	-9
	$\chi^2=2.67$		P=0.45	

The 6 repulsion progenies showed 37 percent crossing over and the 4 coupling progenies 36 percent crossing over. These two crossover percentages are in very close agreement.

Three of the repulsion progenies recorded in table 5 were F_2 progenies segregating for aleurone color. The genetic composition of the parental plants was L_4R^oC/l_4r^rc . The progenies from these plants showed 3:1

ratios of red to non-red stems and 9:7 ratios of colored to colorless aleurone. The repulsion phase of the linkage was exhibited by the stem color segregation and the coupling phase by the aleurone segregation. This peculiar situation is due to the action of the factor R^o which is dominant in aleurone color and recessive in stem color. LINDSTROM (1925) described a similar situation in discussing the linkage of L_2 and R^o . The complete data on these three F_2 progenies are recorded in table 6.

TABLE 6
Data on the F_2 progenies from plants of the composition L_4R^oC/l_4r^oc .

PEDIGREE	COLORED ALEURONE				COLORLESS ALEURONE			
	RED STEMS		NON-RED STEMS		RED STEMS		NON-RED STEMS	
	L_4	l_4	L_4	l_4	L_4	l_4	L_4	l_4
4606-7	41	9	31	2	25	11	7	1
-11	78	13	42	6	74	52	20	4
5775-17	37	13	18	1	30	18	6	0
Totals	156	35	91	9	129	81	33	5

A summary of the data in table 6 to show the repulsion phase of the linkage between L_4l_4 and r^oR^o appears below:

	L_4r^o	L_4R^o	l_4r^o	l_4R^o
Observed	285	124	116	14
Expected, 32 percent crossing over	283	121	121	14
Deviations	+2	+3	-5	0
$\chi^2=0.30$			P=very good fit	

Another summary of these data to show the coupling phase of the linkage between these two factor pairs appears below:

	Colored Aleurone		Colorless Aleurone	
	L_4	l_4	L_4	l_4
Observed	247	44	162	86
Expected, 30 percent crossing over	252	51	152	84
Deviations	-5	-7	+10	+2
$\chi^2=1.77$			P=0.62	

These data indicate a modified 27:9:21:7 ratio. The crossover percentage was computed using a modification of EMERSON'S (1916) general formula described by BRUNSON (1924) for use in trihybrid ratios involving two complementary genes, one of which is linked with a third gene.

The crossover percentages of 32 and 30 computed from these two distributions are slightly lower than those of 36 and 37 computed from the data in table 5. The value of 36 percent has been selected as the most likely value as it is based on coupling progenies and also because it fits better than the lower values certain data presented later in tables 11, 12, 13 and 15.

Tests of l_4 with w_2

The data on the linkage relations between l_4 and w_2 come from 77 F_3 seedling progenies of the cross $L_4L_4W_2w_2 \times L_4l_4W_2W_2$. In as much as these were F_3 progenies, some of them showed the coupling phase of the linkage and others the repulsion phase. The summarized data on these progenies are recorded in table 7.

TABLE 7
Data on the F_3 seedling progenies exhibiting the coupling and the repulsion phases of linkage between l_4 and w_2 .

PEDIGREE	NUMBER OF PROGENIES	GREEN SEEDLINGS	YELLOW SEEDLINGS	WHITE SEEDLINGS
<i>Coupling</i>				
5773	4	396	86	126
5774	7	667	147	253
5775	14	1465	375	569
5776	6	689	164	248
Totals	31	3217	772	1196
<i>Expected, 35 percent crossing over</i>		3140	749	1296
Deviation		+77	+23	-100
•10.21		P=0.006		
<i>Repulsion</i>				
5773	16	1298	542	563
5774	8	425	169	177
5775	17	1720	658	690
5776	5	485	167	176
Totals	46	3928	1536	1606
<i>Expected, 40 percent crossing over</i>		3818	1485	1766
Deviation		+110	+51	-160
$\chi^2 = 21.84$		P=0.00002		

The crossover percentages were computed from a modification of EMERSON'S formula suggested by COLLINS (1924) for use with a 9:3:4 ratio. The data in table 7 indicate 35 percent crossing over between l_4 and w_2 for the coupling progenies and 40 percent for the repulsion progenies.

The poor fit in both the coupling and the repulsion progenies is due largely to the deficiency of white seedlings. This deficiency results from the very close linkage between w_2 and a factor for defective seeds, d_1 . This linkage was reported by LINDSTROM (1923). The defective seeds germinated only 91 percent, whereas the normal seeds germinated 97 percent.

An opportunity to verify the crossover percentages computed from the seedling ratios presented itself in a study of the proportions of the different kinds of F_2 segregations. It will be recalled that l_4 and w_2 came into the cross from opposite parents and the repulsion phase of the linkage would be expected in F_2 . The closeness of the linkage between these two genes will be reflected in the proportions of the various kinds of F_2 progenies from self-pollinated F_2 green plants. The theoretical F_2 populations were computed assuming 35, 40 and 44 percent crossing over respectively and the proportions of the different kinds of F_2 segregations determined.

Table 8 shows a comparison of the observed and expected numbers of F_2 progeny segregations. A total of 146 F_2 progenies is available for study. The χ^2 test for goodness of fit has been determined between the observed numbers of F_2 segregations and those expected with 35, 40 and 44 percent crossing over. The best fit was obtained when 35 percent crossing over was assumed, although each of the crossover percentages given fits well within the limits of chance variations, P in the case of 35 percent crossing over being greater than 0.80.

TABLE 8

Observed numbers of the different kinds of F_2 progeny segregations from F_1 plants of the composition L_4w_2/l_4W_2 and those expected with 35, 40, and 44 percentages of crossing over.

CROSSOVER PERCENTAGE	F_2 SEEDLING SEGREGATIONS			
	BREEDING TRUE FOR GREEN	SEGREGATING FOR 3 GREEN: 1 WHITE	SEGREGATING FOR 3 GREEN: 1 YELLOW	SEGREGATING FOR 9 GREEN: 3 YELLOW: 4 WHITE ¹
Observed	8	28	33	77
Expected, 35 percent crossing over	8	31	31	75
	$\chi^2 = 0.47$		P = greater than 0.80	
Observed	8	28	33	77
Expected, 40 percent crossing over	11	32	32	70
	$\chi^2 = 2.05$		P = 0.56	
Observed	8	28	33	77
Expected, 44 percent crossing over	13	33	33	66
	$\chi^2 = 4.51$		P = 0.22	

¹ Modified by the linkage between l_4 and w_2 .

Tests of l_4 with l_2

The percentage of crossing over between l_4 and l_2 was determined from the F_2 seedling progenies of the cross $L_2l_2L_4l_4 \times L_2L_2L_4l_4$. The complementary interaction of these two factors produced a 9:7 ratio of green to yellow seedlings modified slightly by linkage. The summarized data on 10 F_2 progenies are recorded in table 9.

TABLE 9
 F_2 seedling progenies from self-pollinated plants of the composition L_2l_2/l_2L_2 .

PEDIGREE	NUMBER OF PROGENIES	SEEDLING CLASSES		DEV + P.E. 9:7
		GREEN	YELLOW	
5770 } 5771 }	10	1156	963	2.4
Expected, 9:7		1192	927	
Deviation		-36	+36	
Expected, 43 percent crossing over		1157	962	0.7
Deviation		-1	+1	

The data in table 9 indicate 43 percent crossing over between l_2 and l_4 . The deviation from the numbers expected on the basis of independent inheritance is only 2.4 times the probable error. The deviation however is in the direction of an excess of yellow seedlings which would be expected if the two factors were linked. In view of the usual deficiencies obtained in the yellow seedling classes an excess in this case is of added significance.

Recognition that any deficiency of yellow seedlings would tend to increase the percentage of crossing over when calculated by the formula used (a modification of EMERSON's formula for 9:7 ratios) would suggest, perhaps, that 43 percent was slightly high. Because of that possibility, it was assumed that all kernels not germinated were potentially yellow seedlings. The ratio then became 1156 green and 1012 yellow seedlings from which 37 percent of crossing over was calculated. This value represented the lowest possible percentage of crossing over obtainable from the data at hand, indicating that l_2 and l_4 were linked weakly even by the most conservative estimation.

Forty-three percent crossing over between l_4 and l_2 was decided upon as being more nearly correct. This figure agreed remarkable well with the calculations of crossover values between other factors within the linkage group.

THE LINKAGE GROUP $L_4-L_1, R-W_2-L_2$

LINDSTROM (1925) indicated that the probable arrangement of the genes he studied was $L_1, R-W_2-L_2$. L_1 and R probably are completely linked, and he obtained about 22 percent crossing over between L_1 and W_2 and about 35 percent crossing over between R and L_2 . Data already presented in this paper indicate that L_4 is about 36 units from R , 35 to 40 units from W_2 and 43 units from L_2 . This situation would indicate that L_4 probably is located to the left of R , although if this is the case the distances from L_4 to R and from L_4 to W_2 do not seem to be in very close agreement.

The best proof that this is the real situation could, of course, be had from backcrosses involving l_4 and l_2 or more of the other factors on this chromosome. In as much as 3 of the genes studied (l_2, l_4 and w_2) are lethal, backcrosses were not possible. The best available proof that this is the correct order of these genes comes from data on 2 trihybrid segregations, one involving l_4, R^o and w_2 and the other involving l_4, R^o and l_2 .

The trihybrid $L_4l_4rR^ow_2w_2$

This trihybrid was the result of the cross $L_4L_4rR^ow_2w_2 \times L_4l_4rR^ow_2w_2$. The F_1 plants of this cross would be expected to be of 8 different genotypes $L_4rW_2/L_4rW_2, l_4rW_2/L_4rW_2, L_4rW_2/L_4r^ow_2, l_4rW_2/L_4r^ow_2, L_4rW_2/L_4R^ow_2, l_4rW_2/L_4R^ow_2, L_4rW_2/L_4R^ow_2, l_4rW_2/L_4R^ow_2$. Twelve F_1 plants were self-pollinated. Three of these plants proved to be of the genotype, $l_4rW_2/L_2R^ow_2$. The F_2 progenies from two of them were grown in the field and 135 L_4rW_2 plants (green plants with red stems) were self-pollinated. Some of these plants proved to be homozygous for all 3 of the factors involved, some were heterozygous for 1, some for 2 and some for all 3 of the factors. The F_3 progenies from plants heterozygous for all 3 genes (57 in all) furnish the most critical evidence that the order of the genes is $L_4-R^o-W_2$.

Four different genotypes were expected among the self-pollinated F_2 plants that were heterozygous for all 3 genes. These four genotypes and their F_3 linkage relations are shown below:

	F_2 genotype	F_3 linkage relations
1.	$\frac{l_4rW_2}{L_4R^ow_2}$	Repulsion between l_4 and R^o , and between l_4 and w_2 and coupling between R^o and w_2 .
2.	$\frac{L_4rW_2}{l_4R^ow_2}$	Coupling between l_4 and R^o , between l_4 and w_2 , and between R^o and w_2 .
3.	$\frac{l_4rW_2}{L_4R^ow_2}$	Repulsion between l_4 and R^o , coupling between l_4 and w_2 and repulsion between R^o and w_2 .
4.	$\frac{l_4R^ow_2}{L_4rW_2}$	Coupling between l_4 and R^o , repulsion between l_4 and w_2 and between R^o and w_2 .

Each of the 57 progenies from trihybrid F_2 plants was examined to determine the genotype of the parental F_2 plant. Progenies were obtained representing the first three parental genotypes. If the arrangement of the genes is $L_4-R^o-W_2$ the fourth parental genotype represents the double cross-over combination of these three genes and would be expected to occur less frequently than the non-crossover or single-crossover combinations. No F_3 progenies representing this parental genotype were obtained.

A summary of the data on all of these progenies from which the crossover percentage between R^o and w_2 was computed is recorded in table 10. Data on the coupling progenies indicate 17 percent of crossing over between these two factors and data on the repulsion progenies indicate 13 percent of crossing over. Inasmuch as the numbers were much larger in the coupling progenies the value of 17 percent has been used in computing the expected numbers for the F_3 progenies of the different F_2 parental genotypes. The poor fit in the coupling progenies is due to the deficiency of white seedlings. This value is intermediate between that of 15.4 previously

TABLE 10
Data on the F_3 progenies from F_2 plants of the composition $r^+R^oW_2w_2$.

PEDIGREE	NUMBER OF PROGENIES	NON-WHITE SEEDLINGS		WHITE SEEDLINGS	
		RED STEMS	NON-RED STEMS	RED STEMS	NON-RED STEMS
		<i>Coupling</i>			
5773	15	1540	178	139	385
5774	6	464	55	50	121
5775	28	3337	448	343	777
5776	4	507	81	45	128
Totals	53	5848	762	577	1411
<i>Expected, 17 percent crossing over</i>		5780	669	669	1481
Deviation		+68	+93	-92	-70
$\chi^2 = 29.69$		P = very small			
		<i>Repulsion</i>			
5773	1	107	41	46	1
5774	2	201	98	92	0
5775	1	95	43	41	2
5776	1	91	57	45	1
Totals	5	494	239	224	4
<i>Expected, 13 percent crossing over</i>		485	236	236	4
Deviation		+9	+3	-12	0
$\chi^2 = 0.82$		P = very good fit			

reported by LINDSTROM (1924) between the same two factors and that of 22 reported by him (1925) between L_1 and W_2 .

Unfortunately it was difficult to compute the crossover percentage between l_4 and R^o due to the presence of w_2 . The linkage between w_2 and genes l_4 and R^o resulted in unequal suppression of l_4 classes by w_2 and made any calculation of linkage between l_4 and R^o difficult and uncertain. For this reason the crossover value of 36 percent arrived at from the data in table 5 has been used in computing the expected numbers for the F_2 progenies of the different F_2 genotypes.

Assuming 36 percent crossing over between l_4 and R^o and 17 percent crossing over between R^o and w_2 , a double crossover percentage of about 6 would be expected if there were no interference. The theoretically expected numbers have been computed on the basis of these crossover values.

The parental genotype $l_4r^oW_2/L_4R^ow_2$

With the crossover percentages mentioned above the relative frequency of the gametes produced by this parental combination would be as follows:

Gametic ratio	Gametes	Method of gamete formation
53	$L_4R^ow_2$	Parental type
30	$L_4r^oW_2$	Crossover in the L_4 - R^o region
11	$L_4R^oW_2$	Crossover in the R^o - W_2 region
6	$L_4r^ow_2$	Double crossover
6	$l_4R^oW_2$	Double crossover
11	$l_4r^oW_2$	Crossover in the R^o - W_2 region
30	$l_4R^ow_2$	Crossover in the L_4 - R^o region
53	$l_4r^ow_2$	Parental type

TABLE 11

Seedling progenies from self-pollinated plants of the composition $l_4r^oW_2/L_4R^ow_2$.

PEDIGREE	NUMBER OF PROGENIES	GREENS		YELLOWS		WHITES	
		RED STEMS	NON-RED STEMS	RED STEMS	NON-RED STEMS	RED STEMS	NON-RED STEMS
5773	13	901	131	401	22	113	341
5774	5	234	38	98	6	38	79
5775	17	1469	251	621	37	206	484
5776	1	92	22	36	3	11	33
Totals	36	2696	442	1156	68	368	937
Expected ¹		2687	385	1122	56	441	976
Deviation		+9	+57	-34	+12	-73	-39

$$\chi^2 = 25.71$$

$$P = 0.0001$$

¹ Calculated on the basis that there was 36 percent crossing over between L_4 and R^o , 17 percent crossing over between R^o and W_2 , and 6 percent of double crossing over.

By arranging these gametes in an ordinary trihybrid Punnett square and multiplying them by their relative frequencies, a theoretical phenotypic population can be derived, which should agree with the one observed, provided the linear order of the genes and their distances apart have been correctly assumed. In table 11 are summarized the results from 36 progenies. The agreement between the observed and expected numbers is not very satisfactory due to the deficiency of white seedlings. This deficiency is due to the linkage between w_2 and d_f .

The parental genotype $L_4R^+W_2/l_4R^+w_2$.

Assuming the same crossover percentages as before the gametic ratio for this parental combination would be as follows:

Gametic ratio	Gamete*	Method of gamete formation
53	$L_4R^+W_2$	Parental type
30	$L_4R^+w_2$	Crossover in the L_4-R^+ region
11	$L_4R^+W_2$	Crossover in the R^+-W_2 region
6	$L_4R^+W_2$	Double crossover
6	$l_4R^+w_2$	Double crossover
11	$l_4R^+W_2$	Crossover in the R^+-W_2 region
30	$l_4R^+W_2$	Crossover in the L_4-R^+ region
53	$l_4R^+w_2$	Parental type

Data on 17 progenies from F_2 plants of this genotype are recorded in table 12.

TABLE 12

Seedling progenies from self-pollinated plants of the composition $L_4R^+W_2/l_4R^+w_2$.

PEDIGREE	NUMBER OF PROGENIES	GREENS		YELLOWS		WHITES	
		RED STEMS	NON-RED STEMS	RED STEMS	NON-RED STEMS	RED STEMS	NON-RED STEMS
5773	2	191	16	47	9	26	44
5774	1	103	9	29	2	12	42
5775	11	1031	93	216	67	137	293
5776	3	305	41	74	15	34	95
Totals	17	1630	159	366	93	209	474
Expected ¹		1587	134	383	94	228	505
Deviation		+43	+25	-17	-1	-19	-31

$$\chi^2 = 10.08$$

$$P = 0.07$$

¹ Calculated on the basis that there was 36 percent crossing over between L_4 and R^+ , 17 percent between R^+ and W_2 and 6 percent of double crossing over.

The fit in this case is much better, P being 0.07. There is a deficiency of white seedlings and an excess of green seedlings with non-red stems.

The parental genotype $l_4R^o w_2/L_4R^o W_2$

The expected gametic ratio for this parental combination, with the crossover percentages previously mentioned, would be as follows:

Gametic ratio	Gamete	Method of gamete formation
53	$L_4R^o W_2$	Parental type
30	$L_4r^o w_2$	Crossover in the L_4-R^o region
11	$L_4R^o w_2$	Crossover in the R^o-W_2 region
6	$L_4r^o W_2$	Double crossover
6	$l_4R^o w_2$	Double crossover
11	$l_4r^o W_2$	Crossover in the R^o-W_2 region
30	$l_4R^o W_2$	Crossover in the L_4-R^o region
53	$l_4r^o w_2$	Parental type

The theoretical numbers of each phenotype were calculated in a manner similar to that previously described.

This parental genotype was represented by only four seedling progenies. The data on these progenies are recorded in table 13. The agreement between the observed and the expected numbers was very close, P having a value of 0.54.

TABLE 13

Seedling progenies from self-pollinated plants of the composition $l_4r^o w_2/L_4R^o W_2$.

PEDIGREE	NUMBER OF PROGENIES	GREEN SEEDLINGS		YELLOW SEEDLINGS		WHITE SEEDLINGS	
		RED STEMS	NON-RED STEMS	RED STEMS	NON-RED STEMS	RED STEMS	NON-RED STEMS
5774	2	159	87	42	11	92	0
5775	1	69	37	26	6	41	2
5776	1	69	48	22	9	45	1
Totals	4	297	172	90	26	178	3
Expected ¹		288	162	101	24	186	6
Deviation		+9	+10	-11	+2	-8	-3

$$\chi^2=4.11$$

$$P=0.54$$

¹ Calculated on the basis that there was 36 percent crossing over between L_4 and R^o , 17 percent between R^o and W_2 , and 6 percent of double crossing over.

With the crossover percentages previously mentioned (36 percent between l_4 and R^o , 35 to 40 percent between l_4 and w_2 and 17 percent between

R^o and w_2) there may be some doubt as to whether the order of these genes is $L_4-R^o-W_2$ or $R^o-W_2-L_4$. With only 17 percent crossing over between R^o and W_2 it seems extremely unlikely that the order could be $R^o-L_4-W_2$. With such an arrangement L_4 would have to be closely linked with both R^o and W_2 instead of loosely linked with them.

In order to determine which of the first two arrangements was the more probable the theoretical ratios expected with the order $R^o-W_2-L_4$ were computed for the data in tables 11, 12 and 13. The values of χ^2 then were computed and are shown in table 14 in comparison with the χ^2 values computed for the order $L_4-R^o-W_2$.

TABLE 14
The χ^2 values for the expected ratios in tables 11, 12, and 13 with the gene orders $L_4-R^o-W_2$ and $R^o-W_2-L_4$.

TABLE NUMBER	χ^2 VALUE WITH THE GENE ORDER INDICATED	
	$L_4-R^o-W_2$	$R^o-W_2-L_4$
11	25.71	64.70
12	10.08	43.31
13	4.11	4.27
Totals	39.90	112.28

The χ^2 values in table 14 indicate that the order $L_4-R^o-W_2$ is probably the correct arrangement of these genes.

The trihybrid $L_4l_4r^oR^oL_2l_2$

Additional information indicating that l_4 occupied a locus to the left of R is supplied by the F_2 progenies of the cross $L_4L_4r^oR^oL_2l_2 \times L_4l_4r^or^oL_2L_2$. The F_1 plants of this cross were of several different genotypes. Those heterozygous for all three factors would be represented by the genetic formula $l_4r^oL_2/L_4R^ol_2$. The F_2 progenies from these plants would show coupling between R^o and l_2 and repulsion between l_4 and R^o and between l_4 and l_2 .

It was not possible to compute the crossover percentages between L_4 and R and between L_2 and R from these progenies. Therefore, the value of 36 percent previously determined was assumed between L_4 and R^o and that of 35 percent reported by LINDSTROM (1925) was assumed between L_2 and R^o . On the basis of these two assumed values, and the order

$L_4-R^o-L_2$, about 13 percent of double crossing over would be expected. The expected gametic ratios, therefore, would be as follows:

Gametic ratio	Gametes	Method of Gamete formation
42	$L_4R^o l_2$	Parental type
22	$L_4R^o L_2$	Crossover in the R^o-L_2 region
23	$l_4R^o l_2$	Crossover in the L_4-R^o region
13	$L_4r^o l_2$	Double crossover
13	$l_4R^o L_2$	Double crossover
23	$L_4r^o L_2$	Crossover in the L_4-R^o region
22	$l_4r^o l_2$	Crossover in the R^o-L_2 region
42	$l_4r^o L_2$	Parental type

The factorial interaction of this trihybrid produced a modified 27:9:21:7 ratio of greens with red stems, greens with non-red stems, yellows with red stems and yellows with non-red stems. A comparison of the observed numbers of seedlings in each of these phenotypic classes with the expected numbers calculated according to the gametic ratios assigned is shown in table 15.

TABLE 15

Seedling progenies from self-pollinated plants of the genetic composition $l_4r^o L_2/L_4R^o l_2$.

PEDIGREE	NUMBER OF PROGENIES	GREENS		YELLOWS	
		RED STEMS	NON-RED STEMS	RED STEMS	NON-RED STEMS
5770 } 5771 }	5	423	112	303	112
Expected ¹		404	119	308	119
Deviation		+19	-7	-5	-7

$$\chi^2 = 1.80$$

$$P = 0.62$$

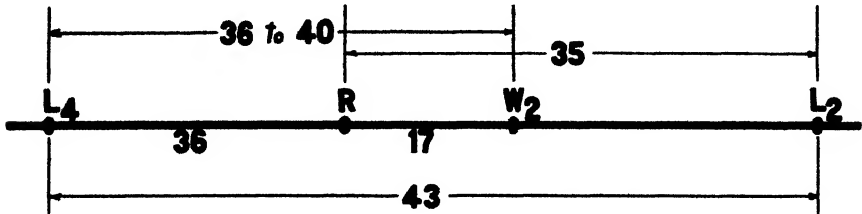
¹ Calculated on the assumption that there was 36 percent of crossing over between L_4 and R^o and 35 percent between R^o and L_2 and 13 percent of double crossing over.

The crossover percentages assumed for computing the expected distribution evidently must be fairly correct as the fit is very good, χ^2 being only 1.80 and P being 0.62.

CHROMOSOME MAP

According to the results obtained in this study, supplemented by work already done by LINDSTROM, who determined the $R-W_2-L_2$ relationship,

the order of the four factors with their approximate crossover percentages is as shown in the diagram below:



The interrelations of these crossover percentages when the double cross-over values are taken into account are in very close agreement. For example, the crossing over between L_4 and L_2 was 43 percent as determined from data involving these two factors only. Crossing over between L_4 and R , and R and L_2 was 36 and 35 percent, respectively, which would mean about 13 percent of double crossovers in the three factor relation L_4-R-L_2 . As double crossing over reduces the actual amount determined between any two factors in a series, the total crossover percentage between L_4 and L_2 should be 43 percent plus twice 13 percent or 69 percent, which agrees very well with 71 percent, the sum of crossover percentages between L_4 and R , and R and L_2 . Another three factor relation, L_4-R-W_2 , furnished similar data. The double crossovers here were calculated to be about 6 percent as the percentages between L_4 and R , and R and W_2 were 36 and 17 percent, respectively. About 35 or 40 percent of crossing over existed between L_4 and W_2 to which amount should be added twice the double crossover percentage, making a total of 47 to 52 percent. The sum of the crossover percentages from L_4 to R and from R to W_2 is about 53 percent. If the correct distance from L_4 to R is 36 units probably the distance from L_4 to W_2 is at least 40 units or more, rather than 35.

SUMMARY

Two new lethal factors for yellow seedlings in maize, both simple recessives, are reported. The allelomorphous factor pairs are designated L_3l_3 and L_4l_4 .

The interaction of these two genes with the two previously reported genes producing yellow seedlings and with certain other chlorophyll genes also is described. Genes l_3 and l_4 are similar to l_2 in their interactions with other chlorophyll factors.

The linkage group to which l_3 belongs was not determined. It showed no indication of linkage with certain members of the $R-G$, $B-L$, $Y-P$, and $Ra-G$ linkage groups.

Gene l_4 showed linkage with 3 members of the $R-G$ linkage group. Its most probable location appears to be 36 units to the left of R .

The order of the four genes of this linkage group included in this study appears to be $L_4-R-W_2-L_2$.

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THE FREQUENCY OF TRANSLOCATIONS PRODUCED BY X-RAYS IN DROSOPHILA

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By the term “translocation” is meant the breaking off of a piece of one chromosome and its attachment to another, non-homologous chromosome. An isolated case of a translocation was encountered in 1918 by BRIDGES (1923). Although involving too small a portion of chromatin to be noticed cytologically by the means available, it was thoroughly demonstrated by genetic means. This case stood alone, however, as a genetic oddity, unparalleled by similar cases that could be satisfactorily proved, until 1926. In that year STERN (1926, 1927) discovered a case in which a portion of the Y chromosome had become attached to the X; this was demonstrated both genetically and cytologically. Somewhat later in 1926, and in 1927, MULLER independently obtained genetic evidence that in his X-ray experiments numerous changes in gene alignment, of varied kinds, including translocations, had been produced (MULLER 1927a); some of these cases too were checked cytologically in 1927 (MULLER 1927b, 1928a, PAINTER and MULLER 1929). It then became of interest to discover with what frequency such translocations between non-homologous chromosomes might arise, as a result of exposure to short wave-length radiation, and of what different types these translocations might be.

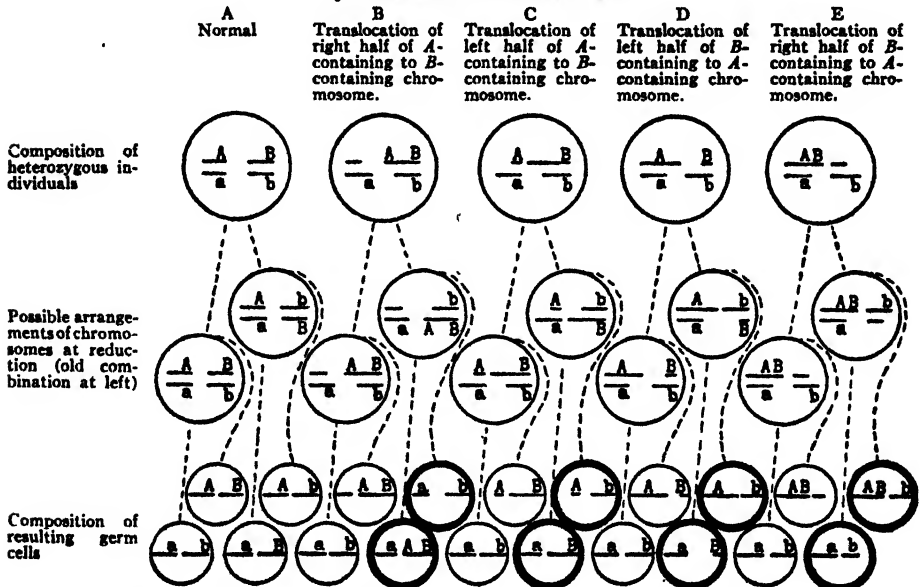
THE METHOD OF ATTACK

In order to investigate this question systematically, crosses were then started of irradiated males by non-irradiated females which differed from the males in a number of "identifying genes" or "markers," located in different chromosomes, and some hundreds of the heterozygous offspring (F_1) were then backcrossed, in individual cultures, so that the distribution of the markers among the progeny of each of them could be determined. Ordinarily, the character differences dependent on different pairs of chromosomes would be distributed at random, according to Mendel's law of "independent segregation," but, if a translocation had occurred, the markers in the chromosomes involved would now appear as though linked.

By way of illustration, suppose that in one pair of chromosomes of the heterozygous F_1 which is to be tested there are the allelomorphic markers A and a , and in another pair B and b , so that the F_1 individual has, so far as these are concerned, a composition which can be represented by $\frac{A}{a} \frac{B}{b}$, (showing homologous chromosomes by parallel lines of different thickness, one below the other). Then, in the normal course of inheritance, the chromosomes might, of course, become arranged at reduction with equal likelihood either in the "old combination" $\frac{A}{a} \frac{B}{b}$, giving germ cells with $\frac{A}{a} \frac{B}{b}$ and with $\frac{a}{a} \frac{b}{b}$, respectively, or in the "new combination," $\frac{A}{a} \frac{b}{B}$, giving germ cells with $\frac{A}{a} \frac{b}{b}$ and with $\frac{a}{a} \frac{B}{B}$, respectively (see figure 1, diagram A). But if there had been a translocation in the sperm cell from which the F_1 had been derived, breaking the A -containing chromosome and tying the piece of it bearing A to the B -containing chromosome, the F_1 composition would be represented by $\frac{A}{a} \frac{B}{b}$, and the chromosomes at reduction could have either the "old" arrangement $\frac{A}{a} \frac{B}{b}$ or the "new" arrangement $\frac{A}{a} \frac{b}{B}$. (If crossing over were possible still other arrangements could occur.) The first arrangement would yield the old combinations of markers $\frac{A}{a} \frac{B}{b}$ and $\frac{a}{a} \frac{b}{b}$, respectively, but the second arrangement would yield germ cells of composition $\frac{b}{b}$ and $\frac{a}{a} \frac{A}{B}$, respectively. The former of these new-combination germ cells (namely $\frac{b}{b}$) and the progeny

resulting from it obviously have a deficiency of the genes in the right half of the originally *A*-containing chromosome, while the latter of the two new combinations carries an excess of genes of this same region. It is therefore likely that one or both of these new combinations will give abnormal and relatively inviable zygotes, or, in the case of a relatively large or potent translocation, that the new combinations will both be fully lethal.¹ Thus, so far as the non-crossover offspring are concerned, at any rate, only the old combinations of markers, *AB* and *ab*, may be found alive among the F_2 , the combinations *Ab* and *aB* being absent, just as though *A* and *B* had been completely linked, or only the old combinations and one new-combination class will appear, or the new combinations may simply be less numerous and show phaenotypic abnormalities.

Figure 1.—Diagram showing nature of combinations produced by normal and by translocated Chromosomes.



(The germ cells in heavy outline contain combinations apt to be lethal to zygotes formed on backcrossing.)

The same result would be just as apt to occur if the "left" end of the left hand chromosome had been attached to the right hand chromosome,

¹ It had been repeatedly insisted upon by MULLER in the earlier *Drosophila* work that a change from the normal proportions ("balance") of genes, caused by an excess or deficiency in the numbers of some genes in relation to the numbers of the others, would tend to produce phaenotypic abnormalities, including inviability. This conception is now fully proved by the present results even as regards disproportions involving the excess or deficiency of *parts* of chromosomes.

as follows: $\frac{A}{a} - \frac{B}{b}$. For, even though A and B are not physically connected here, nevertheless, just as before, the two "new" non-crossover combinations, receiving one of these markers and not the other, will have a deficiency and an excess, respectively, of genes, and will hence tend not to be normal or viable. This result would also follow if either end of the B -containing chromosome should become attached to the A -containing chromosome, or, for that matter, if either the a - or the b -containing chromosome should become broken and have a fragment attached to the other. The failure of one or both new combination classes (Ab or aB) to appear would therefore indicate a translocation between the non-homologous chromosomes containing the markers studied but would not by itself tell us which chromosome was broken and attached to the other nor which end of the broken chromosome had become attached. In some such cases it might even happen, theoretically, that both chromosomes (A -containing and B -containing) had become broken, mutually exchanging parts—a condition recently recognized by BLAKESLEE and BELLING in *Datura* under the term "segmental interchange," but these cases too would give results like the others, in the above tests. The objective of such tests, therefore, is to determine the total frequency of all kinds of translocations between the "marked" chromosomes which disturb the "genic proportions" sufficiently to cause the inviability, or the abnormality, of one or both of the new combinations.

THE INITIAL RESULTS—A STARTLINGLY HIGH FREQUENCY OF TRANSLOCATION

In the first experiment (fall of 1927), the X-rayed males carried in one of their second chromosomes the dominant gene for curly wings (C_y), in the other second chromosome the dominant star eye (S), and in one of their third chromosomes the dominant dichaete (D), thus having the composition

$\frac{X}{Y} \frac{C_y}{S} \frac{D}{\quad}$ (representing the sex chromosomes, the

second pair and the third, in this order, but in the case of the autosomes, labelling only the mutant allelomorphs). They were given the treatment which has been designated as "t4" (MULLER 1928b) and immediately afterwards mated to non-rayed females having the normal allelomorphs of the above genes but carrying attached X-chromosomes with the genes for yellow body, and a supernumerary Y chromosome. The composition of the females might therefore be represented

as follows $\begin{smallmatrix} X_y \\ X_y \\ Y \end{smallmatrix} \begin{smallmatrix} > \\ > \\ \longrightarrow \end{smallmatrix} \underline{\hspace{1cm}} \underline{\hspace{1cm}} \underline{\hspace{1cm}}$. The offspring from this cross which carried the father's X and the mother's Y would be males; those carrying the father's Y and the mother's attached X's would be females. Half the offspring would carry C_v , the rest would carry S . Half would carry D , and the rest its normal allelomorph, but only the D -carrying offspring were used, since a marker in the third chromosome was needed. If, now, we show the chromosomes derived from the rayed father by heavy lines, and again represent only mutant genes in the autosomes, the composition of half of the F_1 males used was $\underline{\underline{X}} \underline{\underline{C_v}} \underline{\underline{D}}$, and of the other half $\underline{\underline{X}} \underline{\underline{S}} \underline{\underline{D}}$. Males of both these types were then crossed with virgin normal females, one male to a culture, and the distribution of sex, curly or star, and dichaete, with regard to each other, among the progeny (" F_2 ") in each of the 90 fertile cultures was examined, to discover any cases in which any of the expected new combinations of the characters in question failed to appear, or appeared in very small numbers. F_1 females were also bred, one to a culture, by normal males. The females which were used had the composition $\begin{smallmatrix} X_v \\ X_v \\ Y \end{smallmatrix} \begin{smallmatrix} > \\ > \\ \longrightarrow \end{smallmatrix} \underline{\underline{C_v}} \underline{\underline{D}}$ and $\begin{smallmatrix} X_v \\ X_v \\ Y \end{smallmatrix} \begin{smallmatrix} > \\ > \\ \longrightarrow \end{smallmatrix} \underline{\underline{S}} \underline{\underline{D}}$; thus these females also might reveal translocations, through counts of the distribution of sex, curly or star, and dichaete, with regard to each other.

It will be seen that the tests of the F_1 males were adapted to reveal translocations involving the rayed X chromosome and the second or third, while the tests of the females would reveal translocations involving the rayed Y chromosome and the second or third. In the case of the Y, however, only cases in which an autosome had been broken and attached to the Y would be discoverable by these methods, not the converse cases of breakage of the Y and its attachment to an autosome, since it is known that excess or deficiency of the Y chromosome or parts of it usually produce no change in viability or appearance.

It should be noted also, concerning the tests of the F_1 females, that D does not serve as an absolute marker for the whole of the third chromosome as it does in the male, since crossing over can occur between it and other parts of this chromosome, in the female. Hence, in the tests of females, some cases of translocations involving the third chromosome may have gone unrecognized, and other cases might be recognizable only by the lessened frequencies of the "new combination" classes rather than by their

complete absence, inasmuch as crossing over might produce viable, genically balanced classes phenotypically simulating the inviable new chromosome-combinations that carry an excess or deficiency of genes. Similarly, crossing over might occur between star and a point of breakage or attachment in the second chromosome. Curly, however, formed a quite satisfactory marker for the whole of the second chromosome, where it was used, since it is associated with an unusual gene-arrangement that undergoes very little crossing over with a normal second chromosome. The close agreement of the results from the female tests with those from the male tests indicates, in general, that few of the translocations were missed in the former which would have been found in the latter.

The numbers of translocations found in this first experiment, and the chromosomes involved, are summarized in table 1, lines 1a and 1b, for the male and female tests separately. In all there were 21 translocations found among the 161 F_1 flies (representing as many X-rayed sperm cells of P_1 tested), or 13 percent. This was a startlingly high frequency, in view of the previous uniqueness of the phenomenon, and was comparable with the rate of detectable gene mutations occurring after X-raying. If the natural translocation rate is similarly high, compared with the natural rate of gene mutation, it must be of widespread, even though *relatively* infrequent, occurrence in nature and may be of importance in evolution.

Examining the sorts of translocations, it is seen that all the possible combinations of the chromosomes studied together were found: namely X-II, X-III, Y-II, Y-III, and II-III (where X-II means a translocation from either X to II or II to X; similarly for X-III, Y-II, etc.). The last class, II-III, was the most numerous, as might perhaps have been expected in consideration of the fact that it involved exclusively the two largest chromosomes. The relative frequencies of the different kinds of translocations will be considered in more detail later, when the results of all the experiments have been presented.

The counts from the individual cases of translocation are shown in table 2. Translocations involving II and III are evidenced by an absence or decided deficiency in numbers of the C_y or S non- D and the non- C_y or non- S D recombination classes. Translocations involving the Y or X and an autosome give results as though either C_y or S or D , as the case might be, had become sex-linked. Thus, if a piece of the second chromosome had become attached to the Y, then the F_1 males, on being bred to normal females, would transmit this piece to their sons, but not to their daughters. The curly (or star) daughters would therefore fail to

develop, since in them the second chromosome would lack the piece, and since the Y carrying the piece attached would also be absent. But in the curly male offspring this piece missing from the second chromosome would be attached to the Y chromosome and they could live. Hence, no curly daughters develop, but curly sons do. The non-curly sons, however, have the piece in triple amount, since one "dose" of it is attached to the Y, and two additional doses are present in their normal positions in the second chromosome pair, one of these normal chromosomes having been the untreated chromosome of the F_1 male, the other having come from the normal mother. On account of the genic disproportion thus produced, the non-curly males would fail to develop. The non-curly females, on the other hand, would not have the piece in triple amount (but simply in double amount) since they receive their father's X (not his Y, with the extra piece). Hence they live. In other words, in the case of a translocation of an autosome to the Y, all, or the great majority, of the flies of one sex show one of the autosomal traits involved, while the flies of the opposite sex show the allelomorph. Similar reasoning applies to an X-II translocation.

As expected, the counts from the tested F_1 females are not as clear-cut as from the males (on account of crossing over). From one of the females (case 15) a batch of progeny was derived in which a double translocation was evident, involving II, III and Y at the same time; here, since II and III gave the same results as though they were tied together, it was not possible to determine whether the translocation to the Y was from II or from III. (This accounts for the caption "sex and II or III" in table 1 at the head of the seventh column).

An additional kind of test was carried out in the case of the cultures derived from the tested F_1 females of this experiment. Here, owing to the method of inheritance imposed by the attached X's the F_2 males carried the Y chromosome that had been present in the rayed sperm of the original P_1 male, and that had been transmitted through the F_1 female of

the composition that has been represented by $\begin{matrix} Y \rightarrow \\ X_r \\ X_r \end{matrix} >$ If this Y chromosome

had had a piece broken off by the X-rays, which had become lost or which had become translocated to a rayed autosome, then, although the F_1 female herself should be fertile (the Y seems to affect the female very little), nevertheless she should give rise to sons (F_2) inheriting the deficient Y, and, as STERN has shown, such males may be sterile. In the case of a complete loss of a fragment of the Y, all the sons (F_2) would be sterile; in the case of a translocation, all the sons receiving the non-radiated autosomes,

and therefore failing to receive the translocated fragment, would be sterile. All the males of the latter class (so far as they could be determined by the markers) were therefore tested, in mass culture, by normal females, in the case of each F_1 - F_2 culture derived from a tested F_1 female, but no instances were found in which all males of the given class proved sterile. Nevertheless, direct tests of individual F_1 males from rayed P_1 fathers crossed by normal (not attached-X) females, showed considerable sterility, as MULLER had previously reported for his experiments. The above tests would hence indicate that most of this sterility in the F_1 males is not due to breakage of the Y chromosome.

CONFIRMATION OF THE FINDINGS, AND DEMONSTRATION OF THEIR CONNECTION WITH THE TREATMENT

A second translocation experiment was then undertaken, involving a cross of X-rayed (t4) S/C_v males by females having the combination of recessive genes in chromosome III termed "IIIpl" (roughoid hairy scarlet pink spineless ebony). The use of the multiple stock here, as well as in the following experiments, had no advantage over the C_v (or S) D stock in getting the translocation frequencies, but was used with a further purpose in mind. The F_1 males in this second experiment carried the radiated Y. Their composition may be represented as $\frac{Y}{X} \frac{S \text{ or } C_v}{\text{IIIpl}}$. These F_1 males were then used as P_2 , being backcrossed, in-

dividually, to females carrying IIIpl or some part of IIIpl (in the combination a, p_x, s_p "theca" referred to below) and the cultures were examined for the presence of recombinations among the progeny (F_2). As table 1, line 2 shows, 13 translocations of type II-III were found, and 3 involving the Y, in a count of 110. In this case, although the dosage factors were so far as possible the same as before, a new machine was used, which later tests have shown to give only a half to a third the effective output, when the controllable factors (time, distance, voltage, amperage, filter and surroundings) are sensibly the same as before. The counts from the translocations are shown in table 2, cases 21-36. In this instance, for some unexplained reason, the total translocation frequency is, if anything, even higher than before, but the disparity between the number of II-III's and those involving the sex chromosome is still evident.

In the second experiment 84 of the 110 P_2 - F_2 cultures were kept track of in such a way that the recency of origin of the translocations found in them could be proved or disproved, that is, the fact that these transloca-

tions had not or had been present in the stock previous to the generation in which irradiation was carried out. This inquiry was undertaken as a substitute for the breeding of controls. The desired end was accomplished simply by recording the P_2 - F_2 cultures in groups, any given group of cultures being "brother-cultures," in the sense that the P_2 males in all the cultures of this group were brothers, having been derived from the same treated P_1 male, and, in fact, carrying a derivative of the same second chromosome of that P_1 male (all cultures of the given group carrying C_v or all carrying S). If, now, a given translocation, recognized from the F_2 count of one of these cultures, had really been present in the original P_1 male before treatment (having been received by him from one of his parents), it would have been transmitted by him to all of his progeny (the F_1 or " P_2 ") of the type used as P_2 in the group. The finding of a certain translocation (Y-II, Y-III, or II-III) in one culture of the group and not in another is therefore tantamount to the finding that this translocation did not arise in any generation antecedent to the P_1 male. Two fertile cultures in a group are enough to prove this point, and the conclusion would still be valid even if both of them contained translocations, if it could be shown that they did not contain the same translocation. The records of these cultures, by groups, are given in table 3.

It will be seen that all of the 12 translocations found in these 84 cultures originated in what would be called the generation of the P_1 male. It is evident that this rate of origination must be far higher than what occurs in untreated material. For while little or no work has previously been done to determine the frequency of occurrence of translocations in control material, still many individual crosses involving genes in separate chromosomes have of course been carried out on *Drosophila*, and if translocations of natural origin occurred at all frequently, a fair number of natural cases should already have been found in the *Drosophila* investigations. If the 12 translocations here in question could have been regarded as an accumulated store, inherited from many previous generations, it might possibly have been contended that they might have been of "natural" origin, but when it is recognized that they must all have originated in one generation (the treated generation) it becomes obvious that the natural frequency of translocation cannot possibly be high enough to account for this, and that nearly all, or all of them, must have been due to treatment. This being the case it was considered unnecessary, for the purpose of the subsequent experiments, to run control tests in them or to keep track of the grouping of the P_2 - F_2 cultures.

were then examined in order to detect the absence of classes having recombinations of the markers in question (considered two at a time). It will be observed that the Y, II, III and IV were subject to study. The experience previously gained in the detection of translocations was now found to be sufficient to enable their detection to be effected without the making of counts, except in special cases.

In experiment 7 the irradiated P_1 flies were males of composition $\frac{(Y)}{(X)}$
 $\frac{ST}{D} =$ (only the mutant genes being represented, as usual.)

Here T represents the gene for truncate wings, which was introduced with an ulterior object in view. So far as the determination of translocations is concerned, T may be disregarded here, since it has little expression in the heterozygous condition unless intensifiers are present in other loci; these were not present in this experiment. The P_1 females were homozygous for e_v , but otherwise normal. The F_1 flies which were used for breeding, as P_2 , were males having the composition $\frac{ST}{D} \frac{D}{e_v}$

(again representing the treated chromosomes with heavy lines). As in experiments 4 to 6, they were backcrossed in individual cultures to recessive females (normal except for eyeless) and recombinations of all the markers, two at a time, were looked for.

The results of each of these experiments are summarized in table 1, lines 4 to 7. In all, 7 translocations involving the fourth chromosome were found, in a count of 420; all of these have been confirmed by later tests. It is practically certain, on theoretical grounds, that all of these cases were transfers from the large autosomes to the fourth. For not only is it probable that breakages of the fourth chromosomes would be very infrequent, as compared with breakages of the larger chromosomes, but, if they did occur, and were accompanied by an attachment of one of the fragments to a non-homologous chromosome, the recombinations carrying only such a small excess or deficiency of chromatin would almost surely be viable, in view of the known fact that individuals with an entire fourth chromosome extra or missing are also viable and not greatly different in appearance from the normal (the "triplo-IV's" and "haplo-IV's" of BRIDGES); hence such cases would probably not have been found by the methods used. Later analysis of the translocations in question has borne out this inference.

Curiously enough, two of the seven translocations to the fourth chromosome occurred in cases of double translocations; that is, in both these

cases two simultaneous translocations took place in the same treated sperm cell of the P_1 male. One of these cases was in experiment 5, where there was a simultaneous translocation between II and III, and to IV from either II or III, it being indeterminable which of the latter had its fragment attached to the fourth, inasmuch as all viable offspring that inherited the radiated II also inherited the radiated III. (This is why this translocation to the fourth had to be recorded as "II or III and IV.") The count of the F_2 cultures showed, instead of 16 classes in approximately equal numbers, only the following: $8C_v D \varphi$, $5 C_v D \sigma$, $5e_v \varphi$, $6 e_v \sigma$, 4 "normal" φ . The "normals" were evidently triplo-IV individuals, caused by non-disjunction of the fourth chromosomes. Such non-disjunction might be expected, theoretically, owing to the large translocated autosomal fragment, through its affinities for its normally placed homologue, interfering with the synapsis and disjunction that should occur between the fourth chromosome to which it is attached and the normal fourth chromosome. The other case was in experiment 4, where the same P_2 - F_2 culture showed both the II-IV translocation and also the Y-III translocation recorded in that experiment. The count in this case gave, instead of 16 classes: $12 S D \varphi$, $12 D e_v \varphi$, $11 S \sigma$, $5 e_v \sigma$. Later tests showed some non-disjunction of chromosome IV to occur in this case also.

Flies inheriting both the Y-III and II-IV translocations which arose in the case last described were then subjected to another irradiation, in order to produce a II-III translocation between these chromosomes, so that a stock giving no recombinations whatever of genes present in the father might be obtained. This end was readily accomplished, one of the desired translocations being found in a batch of 25 P_2 - F_2 cultures from a second irradiation with a t11 dose. Males of the resultant Y-II-III-IV translocation stock, then, always transmit this entire set of chromosomes to their fertile male offspring (though some sterile non-disjunctional males also occur), and these offspring show a similar genetic behavior, as do all fertile descendants in the direct male line. The female offspring, on the other hand, except where non-disjunctional, must always receive the entire homologous set of chromosomes that their father contained, just as though the chromosomes of each haploid set had been joined into a single chain.

In experiments 4 to 7 care was taken not to overlook cases in which one recombination class was absent while the contrary recombination class was present in the F_2 cultures. Three such cases involving II and III were found (1 each in experiments 4, 5, and 6), and they have been included

among the II-III translocations in table 1. In the case in experiment 4 the class (C_9) containing the non-radiated second and the radiated third chromosome was viable; in the cases in experiments 5 and 6, on the other hand, it was the opposite recombination which was the viable one. There is reason, based on analysis of other cases, to believe that when only one of two contrary recombination classes derived from *Drosophila* translocations is viable, that class is usually the "hyperploid," the one carrying the overdose of certain genes, while the lethal recombination is the class having the deficiency of genes, that is, the "hypoploid." If this is true, then the first of the translocations just referred to involved a transfer from II to III, while the other two involved transfers from III to II.

COMPARATIVE FREQUENCIES OF DIFFERENT TRANSLOCATIONS

We may now take count of the comparative frequencies of the translocations involving different chromosome combinations. In experiments 4 to 7 there were 7 translocations from II or III to IV and 8 or 9 translocations from II or III to Y (transfers in the opposite direction being excluded in the case of both these kinds of translocations, as previously explained). It therefore seems as though fragments of the long autosomes were about as likely to become attached to the small fourth as to the Y chromosome. When, however, we examine the results of the other experiments involving the Y, we find a somewhat higher frequency than in experiments 4-7 of the Y-II and Y-III translocations, when the latter are compared with the frequency of II-III translocations as a standard of reference. It may be, therefore, that the observed frequency of Y-II and Y-III translocations in experiments 4-7 was the result of a chance fluctuation that caused it to be somewhat below the mean value. Since the II-IV and III-IV frequencies would not necessarily fluctuate in the same direction it is possible that the translocations to the Y are really somewhat more numerous than to chromosome IV; this difference in frequency, if it exists at all, cannot be very great, however, and certainly is not proportionate to the difference which exists between the physical sizes of these chromosomes, as cytologically observed.

In order to compare the frequencies of translocations involving the Y and the X, respectively, we may again find each frequency in terms of its relation to the frequency of II-III translocations found in the same experiment, using the latter as our standard of reference. From table 1, on the line marked "Total for X, II, III," we find that there were 8 translocations of types X-II plus X-III, as compared with 18 of types II-III, that is, a ratio

of 1:2.2. On the line below this we find that there were 17 (or 18) translocations of types Y-II plus Y-III, as compared with 67 of type II-III, a ratio of 1:3.7. Thus these figures indicate a higher (apparently nearly twice as high) incidence of translocations involving the X than of translocations involving the Y. The absolute number (8) of X translocations found is, however, too small for us to be certain of the "significance" of such a difference in frequency.

Such a difference as above indicated would be expected if the translocations of types "X-II" and "X-III" found include translocations in both directions (from II to X and also from X to II, etc.). For, as previously stated, the translocations found involving Y were always in the same direction—from the autosome to the Y, those from the Y to the autosome having remained undetected. However, there is evidence that a somewhat similar limitation applies also in the case of the X, when the present methods of detection are employed. For, as we shall see later, breeding tests have indicated that a translocation is usually accompanied by the appearance of a recessive lethal in the chromosome which underwent breakage. Thus a broken X, received by an F₁ male, would usually cause the death of the latter, even though both fragments were present. It was the F₁ males, (derived from crosses of radiated P₁ males by attached-X females) which were used (experiments 1a and 3) in tests of translocations involving the X chromosome. Hence classes "X-II" and "X-III" probably represent almost exclusively translocations in the direction "II to X" and "III to X." The three translocations involving the X which appeared in experiment 1a were analyzed genetically, and all of them proved to be in this direction. Translocations in the opposite direction can occur, for in other experiments of MULLER, in which females containing irradiated X's were tested, translocations in the direction, from the X to the autosome, have been found; these in most, but not all, cases have proved lethal in the male.

If, then, we consider our present classes "X-II" and "X-III" as being in only one direction, the difference in frequency between these and the Y translocations appears more pronounced, and the difference in frequency between the translocations involving the X and those involving IV cannot be due to "fluctuations of sampling" (8 translocations to X in an experiment yielding only 18 of type II-III, and 7 translocations to IV in an experiment yielding 48 of type II-III). The results will, however, be less equivocal in an experiment in which all types can be studied at once, since there is some indication that the frequencies of different types of translocations, relative to one another, may also be subject to variation due to determinate causes.

Regardless of our conclusions concerning the frequencies of the translocations to X, to Y, and to IV, respectively, relatively to each other, there is no room for doubt that the frequency of each of them is very significantly lower than the frequency of type II-III. Since class "II-III" is in reality composed of the translocations from II to III plus those from III to II, it is legitimate to compare its frequency with that of the sum of X-II and X-III or the sum of Y-II and Y-III, or the sum of II-IV and III-IV, inasmuch as any single one of these other classes comprises translocations exclusively, or nearly exclusively, in one direction, namely, from the larger to the smaller chromosome, being in this respect unlike II-III. As table 1 shows, there were, in experiments yielding 18 of class II-III, 8 of classes X-II plus X-III; there were, in experiments yielding 67 of class II-III, 17 or 18 of classes Y-II plus Y-III; and there were, in experiments yielding 48 of class II-III, 7 of classes II-IV plus III-IV. These give ratios of 1:0.45, 1:0.27, and 1:0.15, respectively. In brief, then, the figures 1:0.45:0.27:0.15 represent the relative likelihoods, as indicated by our present data, of a fragment from a long autosome becoming attached to the other long autosome, to the X, to the Y, or to the fourth chromosome, respectively.

The cause of this variation in the likelihood of attachment to the different chromosomes is still obscure. Light may be shed upon it through more detailed analysis of the structures of the individual translocations; this is a project now well under way. If, for example, a considerable proportion of the translocations were mutual, involving breakage of and transfer from and to both chromosomes concerned at once (BLAKESLEE's "segmental interchange"), it would follow that (so far as these mutual translocations were concerned) their frequencies might be roughly proportional to the products of the lengths of the two chromosomes taking part (supposing that the chance of breakage in a chromosome is roughly proportional to its length). As yet, however, the evidence seems against most of the translocations in *Drosophila* being mutual. Again, we might account to some extent for the apparent relation between the length of a chromosome and the likelihood of its serving for the attachment of a fragment from another if the attachment point could be anywhere along its length, rather than always terminal. This would not necessarily require the fragment to become attached to the side of a chromosome—a process that might lead to difficulties in its growth and longitudinal division. It might also come about if the "recipient" chromosomes sometimes underwent a breakage simultaneous with that of the "donor" chromosome, accompanied by reunion of the pieces in a different arrangement from that of before ("inversion" or "in-

section"), one or both of the breakage points thus serving for the attachment of the fragment from the donor.

Neither mutual translocation nor any kind of mutual breakage, nor side attachment, can well be the rule, however, else we should expect a much greater disparity between the different classes of translocations than actually exists. For the figures 1.0:0.45:0.27:0.15 by no means express the relative lengths of the chromosomes respectively serving as recipients; these are more nearly given by the figures 1.0:0.67:0.67:0.01. The products of the lengths show still greater disparities. In various cases already analyzed, moreover, it has been proved that the recipient chromosome underwent no breakage or rearrangement, and that the attachment was terminal, though mutual translocations have also been found.

Still other problems would be raised if it should be found that the relative frequencies of the different kinds of translocations differ in different experiments. For example, a comparison of the figures for experiments 5 and 6, in table 1, suggests that in the former there were significantly fewer translocations to Y and IV, in proportion to the II-III translocations, than there were in the latter. If such differences were to be substantiated, we could no longer use the II-III class as a fixed standard of reference, and our relative figures would have meaning only when considered in connection with certain other determining conditions in the experiment, of a nature at present unknown.

THE EFFECT OF TRANSLOCATIONS WHEN HOMOZYGOUS

Reference has been made in the foregoing to the finding that the majority of the translocations were lethal when homozygous. Experiments 3 to 6, inclusive, were purposely framed in such a way that no lethals (for example, C_y , S or D , which are all lethal when homozygous) would be in the radiated chromosomes to begin with; these therefore would not prevent the obtaining of the translocated chromosomes in homozygous condition. Various specimens of translocations, of different kinds, involving X, Y, II, III, and IV, have now been sampled, in tests constructed for the purpose of getting them homozygous. Relatively few could be obtained homozygous, and those that could have usually shown sterility and different kinds of morphological abnormalities, when homozygous, just as though they contained a gene-mutation or as though, like the hyperploid and hypoploid types, they were genically disproportioned. The detailed evidence on these points must be reserved for another paper.

Three possible explanations present themselves for this at first sight surprising situation.

(1.) There has been a simultaneous gene mutation (these are usually

lethals) at some other locus in the chromosome, at a distance from the locus of breakage or attachment. Such a coincidence, occurring in the majority of cases, seems highly unlikely. However, there is some as yet inconclusive experimental evidence that one genetic change tends to be associated with another elsewhere in the same cell, so we can not abruptly dismiss this possibility as yet.

(2.) The alteration in intermolecular surroundings of the genes directly adjacent to the points of breakage and reattachment, in other words the alteration in intergenic contiguities, has in itself brought about a change in the quantity or quality of the physico-chemical action of these genes upon the protoplasm, so as to make them, in effect, somewhat different genes, as though gene-mutations had taken place in the genes on either side of the breakage and attachment points. Plausibility is lent to such an assumption through STURTEVANT'S finding that two genes for bar eye adjacent to one another in the same chromosome seem to have an amount of effect on the development of the eye different from that of two otherwise identical genes for bar eye that lie in separate, homologous chromosomes. Fundamental problems of genetics are involved here which merit active prosecution.

At present we have at least one ray of light on the point here at issue. If this assumption is true, then the genes next to the attachment point on the recipient chromosome should become changed in their action just as much as the genes next to the breakage point on the donor chromosome. Nevertheless, in the cases where there was the possibility of distinguishing such an effect—the cases of translocation to the X, 3 or 4 of which have been studied in some detail, and in which the male serves to reveal any changes in genes of the X—no lethal action or visible abnormalities could be detected. It is true that these may only count as one case in point, since the attachment was in each instance to the right hand end of the X and it might be assumed that this was for some reason a locus particularly insensitive to the sort of change in question. However, till further evidence is forthcoming,* these cases must be taken as opposed to the present interpretation.

(3.) There is finally the possibility that in the act of breakage a chromosome usually becomes injured, so that one or more of the genes next to the breakage point either become destroyed, or are caused to mutate in some way. (Not that they are made functionally different by their changed relationships, as in possibility 2, in which case a reestablishment of the original alignment would automatically restore the original genotype

* More light on the question will be obtained when a study is made of the effect of attaching extra segments to the end of X chromosomes which have, through previous gene rearrangement, acquired new terminal points.

exactly, but that they have been permanently altered by some "injurious" process which accompanied the breakage, but which was not inseparable from the latter.) By a process of elimination, this possibility seems for the time being the most probable. It agrees in general with BRIDGES' interpretation of why his original translocation was not viable when homozygous—namely that the attached piece did not represent quite all of the originally detached piece, a small portion next to the breakage point having become lost in the process of breakage or transfer. It is not, however, necessary to suppose that the small portion was lost; it may merely have been caused to mutate. A breakage, if it could occur in the middle of a gene, instead of between genes, would be bound to cause such a loss or at least mutation; this possibility merges into that of alternative 2. If the genetic material be regarded as a continuum, instead of as segmentally arranged in units, the genes, there would be no distinction between an intra-genic and an inter-genic break, and all breaks would partake of the nature of gene-mutations, as postulated in 2. Reasons have, however, been given (MULLER 1926-1929) for regarding the genetic material as segmental.

The above being the apparent possibilities, they must be kept in mind so that as the evidence concerning translocations accumulates it may be considered constructively, in its bearing on these questions, and also so that new methods of experimental attack on them may if possible be evolved. But no matter which of the possible causes, if any, should turn out to be correct, one apparent effect of the phenomenon in question is so obtrusive that it cannot be ignored here. That is, since most translocations are found to be lethal when homozygous, and the remainder seem commonly to be sterile and abnormal, it would at first sight seem highly unlikely that translocations could play a role in the evolutionary processes occurring in nature. For the establishment of the ordinary translocation in homozygous condition, in a group of individuals, would be tantamount to exterminating the group.

The situation is, however, not so impossible as it might seem at first sight. If the translocations are accompanied by gene mutations, or by genetic changes similar in their effect to gene mutations, it is only to be expected that the majority of them all would be lethal, and that, of the remainder, which were not lethal, the majority would be antagonistic to survival and reproduction. For, as is well known, that is true of the gene mutations themselves, and it is just what is to be expected of any random changes occurring in a complex organization. Nevertheless, evolution must have proceeded through gene mutations, that is, through the very rare gene mutations which happened not to be detrimental, and which there-

fore could withstand the test of natural selection. The numerous lethals, which perished, were thus only a necessary concomitant of the same process which produced the relatively few mutations of a non-detrimental character. The same may therefore be true also of translocations and other changes in gene alignment. In fact, the somatic or physiological changes commonly accompanying translocations may from this point of view be considered as an aid to their becoming established in a species, for then there might be a few adaptive translocations which would have the aid of natural selection in spreading through a group of the species. On the other hand, if the translocations never had any somatic expression their spread would occur only under extraordinary accidental circumstances, as through a series of successive decimations of a population, in each of which only a few chance-chosen individuals survived; undoubtedly, however, such events happen more than once in most groups, in the course of thousands of generations.

THE FREQUENCY OF TRANSLOCATIONS AS COMPARED WITH
THAT OF GENE MUTATIONS.

Although the survival of translocations, provided they occur, thus presents no insuperable obstacles, the question of their frequency of occurrence remains to be considered. If their frequency is far below that of ordinary gene mutations we should expect far fewer translocations than gene mutations to become incorporated in a stock in the course of its evolution during a given period. Evidence derived from a comparison of the cytology, and of the genetic maps, of related species of *Drosophila*, indicate this to be almost certainly true [see the work of METZ (1914, 1916) and of STURTEVANT 1921, STURTEVANT and PLUNKET 1926], though *some* changes in gene alignment, both inter- and intra-chromosomal, must undoubtedly have occurred since the origination of the genus. Direct experimental evidence on the present question is lacking, since no systematic searches for translocations in control material, similar to the mutation frequency studies of the authors, have been made. If, however, many of the mutations in somatic characters which have been observed in untreated material had been accompaniments of translocations or of other gene rearrangements, considerable positive evidence of this should, it seems, already have been adduced in all the locus determinations which have been made of the *Drosophila* mutations. And such evidence has not so far appeared.

From the present studies, on the other hand, we may obtain some direct evidence regarding the frequency of translocations induced by X-rays, as compared with gene mutations similarly induced. In all the data here

presented, together, there was a total of 117 translocations found, each of which involved at least one breakage of one of the long autosomes, and its attachment to some non-homologous chromosome or other in the same cell. There would probably have been about 8 more such translocations found, if chromosome IV had been studied in all the experiments, thus making a more complete figure of 125. These were distributed among a total of 883 tested F₁-F₂ cultures, from P₁ males given an average X-ray dosage of "t9" (in terms of the newer machine). The frequency of translocations from both of the long autosomes was therefore 125/883 or 14 percent. Thus we must conclude that a given one of the long autosomes, subjected in the sperm cells to treatments of about t9 strength, becomes broken and translocated to some non-homologous chromosome—X, Y, IV, or the other long autosome—in approximately 7 percent of the treated sperm cells. (Correction is not made here for mutuals or for possible differences in frequency to X and to Y.)

This figure of 7 percent for a "t9" dose may then be compared with the figure 12 percent, which represents the number of detectable "mutations"—mainly lethals—found by MULLER in 1926 and 1927 in the X-chromosome of sperm cells given the equivalent of a "t12" dose ("t4" on the old machine) (MULLER 1928b), or with the figure 8 percent, the percentage of X-chromosome lethals found by HARRIS this year (HARRIS 1929), following a "t8" (new machine) treatment of the sperm. It must, however, be remembered that the long autosomes are about one and a half times as large as the X, both cytologically and genetically. In a given portion of a long autosome about the size of the X, then, we should find only about $\frac{2}{3} \times 7$ percent, or 4.7 percent of breakages accompanied by translocations, with a t9 dose, or, if the effect is proportional to dosage, about 0.5 percent with a t1 dose. This, then, is to be compared with 1.0² percent of visible plus lethal "mutations" in the X-chromosome per each "t1" of dosage. The rate of translocations detectable by the present methods in the space of a "unit" in one of the long autosomes is therefore just about half the rate of "mutations" detectable by the methods used for these, in about the same space on the X-chromosome. These "mutations," however, themselves included a considerable proportion of chromosome abnormalities. It is likely, moreover, that at least half of the translocations which occurred escaped detection. This follows from the fact that any particular fragmentation breaks the chromosome into a fiber-bearing fragment (the longer fragment, in the case of the long centrally attached autosomes), and a fragment without fiber attachment. If now the fiber-bearing fragment of a chromosome became attached to another

² Oliver's recent work shows 1.25 percent to be the more nearly correct figure here.

chromosome, an abnormal chromosome, having two points of fiber attachment, would become formed, while the other fragment, lacking any fiber, would fail to be transported in cell division, and an inviable offspring would probably ensue. The real rate of occurrence of translocations may therefore be just as high as, or higher than, that of gene mutations, after X-ray treatment has been applied to the sperm cells.

This conclusion is rather surprising, if, as now seems likely from the work of HANSON (1929a) and of OLIVER (1930), the genetic effectiveness of X-rays remains proportional to dosage, and if in addition natural mutations and translocations should be mainly due to "natural X-rays." For in that case we should expect as many natural translocations to occur as natural detectable "point mutations." We can be virtually sure that this is not the case. Owing to the property which translocations have of being usually accompanied by lethal or other detectable phenotypic effects, a large number of them should have been found in the work of discovery and analysis of apparent gene mutations in *Drosophila*, if the translocations were really comparable in numbers to the gene mutations, even though translocations as such had not been specifically looked for. The absence of cases in the earlier work thus indicates a real scarcity of them, as compared with the frequency of ordinary gene mutations. There can, however, be no doubt about the high frequency of the translocations artificially induced in the present experiments, and the fact that this frequency must have been comparable with that of the gene mutations here. How then shall we reconcile these two conflicting series of findings?

It must be remembered that the natural cases dealt with represent a sum total of the mutations that have occurred at all different stages of the life cycle, and in either sex, indiscriminately. All the induced translocations and gene mutations considered, on the other hand, occurred at one restricted stage—namely, in the mature spermatozoa. There is some evidence, from work of HANSON (1929b) and of HARRIS (1929), that the germ plasm at this stage is peculiar in its sensitivity to radiation. At least, considerably more of both gene mutations and chromosome abnormalities occur in offspring from cells radiated in the spermatozoan stage than in those from other rayed cells; conceivably, however, this may be due to a higher selective death rate of the mutant cells in stages other than that of the spermatozoa. Whether the gene-mutation frequency or the chromosome-abnormality frequency is raised more has not as yet been determined. However, work of MULLER (as yet unpublished) on the relative

TABLE 1

NUMBER OF EXPERIMENT	X-RAY MACHINE USED	X-RAY DOSE/DGE	SEX OF P ₁ TESTED	TOTAL FERTILE CUP-TUNES	NUMBERS OF TRANSLOCATIONS INVOLVING:						CHROMOSOMES STUDIED
					SEX AND II	SEX AND II OR III	SEX AND III	II AND III	SEX AND II AND IV	II OR III AND IV	
1	old	t4 ^a	♂	90	2		1	6			X II III
2	new	t4 ^b	♀	71	3	1	2	6			Y II III
3	"	t8	♂	110			3	13			Y II III
4	"	t8	♂	192	2	1	2	12	1		X II III
5	"	t12	♂	44			1	4			Y II III IV
6	"	t8	♂	172	(1?)		1	23	3	2	Y II III IV
7	"	t8	♂	159	3		1	15			Y II III IV
			♂	45	2			6			Y II III IV
Total for X, II, III (1a; 3)				282	4	1	3	18			X II III
Total for Y, II, III (1b, 2, 4, 5, 6, 7)				601	8(+1?)	1	8	67			Y II III
Total for II, III, IV (4, 5, 6, 7)				420				48	4	1	II III IV
Entire Total				883	12(+1?)	2	11	85	0	4	(X or Y) II III (IV).

Among the cultures in experiment 5 were 2 in which all the offspring (F₂) contained the nonradiated second chromosome, bearing Curly. There were 28 offspring in each of these cultures. Evidently a dominant lethal had been induced in chromosome II; the viability of the P₁ male containing it may have been due to its not having contained the lethal in all tissues of its body (owing to the fractional effect often shown by mutants in the first generation after radiation of the sperm) or it may have been due to an incompleteness of action of the lethal (the latter then being really a "semi-lethal"). One clear case of a dominant semilethal induced in chromosome II, that produced a disarrangement of the bristles, was also encountered in experiment 5, and one dominant semilethal, producing various thoracic abnormalities, was found associated with a II-III translocation yielding a count of 21 C, D and 2 non-C, non-D (variants).

A "delta"-like vein abnormality, located in the radiated third chromosome, was found in one of the cultures of experiment 6 not involving a translocation.

TABLE 2
Progeny counts in F₂ cultures involving translocations and other abnormalities.

EXPERIMENT NUMBER	CASE NUMBER	SEX OF P ₁ TESTED	SEX CHROMOSOMES TESTED	MARKERS USED IN			NUMBERS OF PLANTS RECEIVING RADIATED CHROMOSOMES AS INDICATED BY MARKERS (<i>S_x</i> = radiated X or Y)						TYPE OF AB-NORMALITY	REMARKS
				II	III		<i>S_x</i>	—	<i>S_x</i> II	<i>S_x</i> III	II	III		
1.	1-6	♂	X	S or C _y	D	✓	✓	✓	0	0	0	0	II-III	{ small piece or much non-disjunction
"	7	"	"	"	"	✓	0	✓	0	0	0	✓	X-II	
"	8	"	"	C _y	"	36	0	32	28	0	15	19	"	
"	9	"	"	"	"	11	6	10	11	0	0	0	X-III	
"	10	♀	Y	"	"	14	10	24	0	0	0	0	II-III	{ some crossing over between D and translocation
"	11	"	"	"	"	23	10	14	25	0	0	0	"	
"	12	"	"	"	"	17	15	12	0	0	0	0	"	
"	13	"	"	"	"	14	6	23	11	0	0	0	"	
"	14	"	"	"	"	12	23	33	5	0	1	3	"	
"	15	"	"	S	"	8	1	0	8	1	0	0	Y-II-III	
"	16	"	"	"	"	8	0	0	14	17	0	0	Y-II	{ double translocation; some non-disjunction and crossing over
"	17	"	"	C _y	"	11	1	1	9	15	1	1	"	
"	18	"	"	"	"	9	1	2	5	4	0	0	"	
"	19	"	"	"	"	9	0	0	9	0	7	2	"	
"	20	"	"	"	"	15	3	8	15	9	16	10	Y-III	{ some non-disjunction
2.	21	♂	"	S	III pl†	22	17	10	18	0	0	0	"	
"	22	"	"	"	"	25	14	1	10	0	0	0	II-III	
"	23	"	"	"	"	17	26	8	6	0	0	0	"	
"	24	"	"	C _y	"	18	16	8	9	0	0	0	"	{ much crossing over
"	25	"	"	"	"	15	11	11	10	0	0	0	"	
"	26	"	"	S	"	8	13	12	13	6	7	0	"	
"	27	"	"	"	"	3	3	4	0	0	0	0	"	
"	28	"	"	"	"	14	11	15	14	0	0	0	"	{ confirmed in further tests
"	29	"	"	"	"	9	17	11	11	0	0	0	"	
"	30	"	"	"	"	8	13	6	14	0	0	0	"	
"	31	"	"	"	"	22	14	24	13	0	0	0	"	
"	32	"	"	C _y	"	20	16	27	18	0	0	0	"	{ hyperdiploid classes viable
"	33	"	"	"	"	10	10	16	20	0	0	0	"	
"	34	"	"	S	"	14	0	0	15	0	15	20	Y-III	

TABLE 2. (Continued)
Progeny counts in F_2 cultures involving translocations and other abnormalities.

EXPERIMENT NUMBER	CASE NUMBER	SEX OF P ₁ TESTED	SEX CHROMOSOMES TESTED	MARKERS USED IN		NUMBERS OF FLIES RECEIVING RADIATED CHROMOSOMES AS INDICATED BY MARKERS (S _x = radiated X or Y)							TYPE OF ABNORMALITY	REMARKS	
				II	III	S _x	—	S _x II	II	S _x III	III				
2	35	♂	Y	S	IIIpl	1	0	0	1	0	3	5	0	Y-III	confirmed in further tests
a	36	a	a	C _y	a	2	0	0	2	0	7	6	0	a	a
3.	37	a	X	"aps	theca ¹ †	10	10	37	53	0	0	0	0	II-III	multiple-mutants have low viability in this experiment
a	38	a	a	a	a	2	8	22	17	0	0	0	0	a	
a	39	a	a	a	a	3	11	13	25	0	0	0	0	a	a
a	40	a	a	a	a	17	15	47	60	0	0	0	0	a	a
a	41	a	a	a	a	28	26	21	37	0	0	0	0	a	a
a	42	a	a	a	a	27	29	33	49	0	0	0	0	a	a
a	43	a	a	a	a	16	12	28	30	0	0	0	0	a	a
a	44	a	a	a	a	9	12	16	27	0	0	0	0	a	a
a	45	a	a	a	a	6	7	37	47	0	0	0	0	a	a
a	46	a	a	a	a	9	10	29	27	0	0	0	0	a	a
a	47	a	a	a	a	10	16	25	32	0	0	0	0	a	a
a	48	a	a	a	a	10	0	0	47	0	0	0	0	X-II-III	double translocation some non-disjunction
a	49	a	a	a	a	9	1	1	34	14	1	0	18	X-II	
a	50	a	a	a	a	1	0	0	7	13	0	0	17	a	a
a	51	a	a	a	a	5	0	0	49	0	11	21	0	X-III	a
a	52	a	a	a	a	6	0	1	17	0	23	12	0	a	a
a	53	a	a	a	a	0	0	10	14	0	0	14	17	dominant lethal in II	a
a	54	a	a	a	a	1	0	24	32	0	0	0	0	dominant lethal and	or contamination; not counted as translocation
a	55	a	a	a	a	0	0	72	67	0	0	0	0	II-III	

* The symbol $\sqrt{}$ indicates that some flies of class indicated were present in each of the cultures in question, but number was not recorded.

† "aps theca" = $a, p_x, l_A, s, e^+ r_o, c_a$.

‡ The markers "III pl," "aps" and "theca" were present in the non-radiated P_1 eggs; the markers $C_y, S,$ and D , when used, were present in the radiated P_1 sperm.

effects of radiation on immature female germ cells and on spermatozoa indicates that the chromosome-abnormality frequency is increased much more in the spermatozoa as compared with the oögonia, than is the gene-mutation frequency. It is therefore possible that the high rate of translocations, as compared with gene mutations, observed in the experiments reported in the present paper, is a phenomenon especially pronounced in the spermatozoa.

Although the above consideration might partially account for the discrepancy in question, it is probably by no means sufficient to explain all of it, for undoubted displacements of chromosome sections have been found following irradiation of other cells than spermatozoa. It is therefore likely that there is a real difference between X-rays and the "natural" cause or causes of mutations, in regard to their relative effectiveness in altering the genes and in causing breakages and reattachments of parts of chromosomes. This conclusion is strengthened by the evidence, obtained by MULLER and MOTT-SMITH since the present paper was written, that natural radioactivity is not the cause of most mutations in untreated *Drosophila*.

SUMMARY

1 a. Methods are described for detecting the occurrence of translocations in *Drosophila*, based upon the principle that the effect of genic disproportion (excess or deficiency) involving even sections of chromosomes will often be sufficient to cause the inviability or abnormal appearance of one or both recombination classes of zygotes.

b. The results obtained by the use of these methods amply demonstrate the validity of the theoretical conceptions upon which they are based.

2 a. The frequency of translocations following heavy irradiation by X-rays has been found to be surprisingly high. In all, in the experiments herein described, 117 translocations, all or nearly all of them involving at least one breakage of one of the long autosomes and the attachment of a fragment of it to a non-homologous chromosome, have been found in a total of 883 fertile P_2 - F_2 cultures derived from irradiated P_1 males.

b. The percentage of such breaks and reattachments of a given long autosome is thus at least 7 percent. Unit for unit of the chromosome, and dosage for dosage, this is just about half the rate of occurrence of detectable mutations of all kinds, as found in the X chromosome.

c. When allowance is made for the fact that translocations involving the attachment of the fiber-bearing instead of the fiberless fragment are not included in the above figure of 7 percent and that translocations too small to

EXPLANATION OF TABLE 2

This table can be explained by considering specifically experiment No. 1, cases 1-6. The sex of the F_1 fly tested was male, as indicated in column 3. The sex chromosome tested, as indicated in column 4, was the X, because the radiated father was mated to an attached X female; he therefore transmitted his X (rather than his Y) to his F_1 male offspring. The markers used in chromosomes II and III, as indicated in columns 5 and 6, were *S* (star) or *C_y* (curly), for chromosome II, and *D* (dichaete) for chromosome III. These genes are dominants. The F_1 male receives two of them, *S* (or *C_y*) and *D* from his radiated male parent. He receives the normal allelomorphs, *s* (or *c_y*) and *d*, from his normal, unirradiated mother. In the F_1 males, sperm cells of four classes (so far as these markers are concerned) are formed by Mendelian segregation, namely, *SD*, *Sd*, *sD*, and *sd* (or *C_y* in place of *S*), and these contain, respectively, the radiated II and III chromosomes, the radiated II and unirradiated III, the unirradiated II and radiated III, and the unirradiated II and unirradiated III. When these F_1 males are mated to normal females, offspring (F_2) that receive the radiated II and III chromosomes will appear *SD* (that is, star dichaete). Those that receive the radiated II but the normal III will appear *Sd* (star, not-dichaete), etc. Column 7 in the table is headed by the caption *S_x* II and III, and experiment 1, cases 1-6, are checked for these chromosomes; that is, there appeared some flies in the F_2 that were star and dichaete, and that therefore received II and III from their radiated grandparent. Being male, they also received a sex chromosome (*S_x*) from him. The next column is headed II and III, and below it is a check. This means that some flies appeared that received II and III from their radiated grandparent, but that, being female, they did not receive his radiated sex chromosome, as indicated by the absence of the symbol *S_x* in this column. The ninth column is headed "*S_x*" and again is checked below. This means that some flies appeared which received *S_x*, but neither II nor III from their radiated grandparent. The dash in the following (tenth) column, with a check below, indicates that some F_2 flies appeared that had not received *S_x* or the II or III from their radiated grandparent. In the eleventh column, headed *S_x* II, there is an O. This indicates that no flies appeared in the F_2 that received the sex and the II, but not the III, from the radiated grandparent. In the twelfth column, headed simply by II, there is also an O, showing that flies which failed to receive the radiated sex chromosome, but received the radiated II, and not the radiated III, likewise were missing. Columns 11 and 12, taken together, thus show that, regardless of the sex chromosome, flies with II but without III from their radiated parent could not live. In a similar manner, columns 13 and 14 indicate that flies that received only the III chromosome of their radiated grandparent, and not his second also, could not live. In other words, in experiment 1, cases 1-6, flies that received II without III, or III without II, could not live and did not make their appearance in the F_2 . This fact indicates a translocation from II to III or from III to II, and is accordingly recorded as such in column 15, under the heading "Type of abnormality." In the remaining experiments, after case 7, (namely, experiments 1, 2 and 3, cases 8 to 55), the various viable classes of F_2 offspring were counted, instead of being simply "checked" for the appearance of a fairly large number of offspring belonging to these classes. The counts are indicated in columns 7 to 14.

result in readily detectable effects of the genic disproportion also are not included, whereas all kinds of chromosome abnormalities as well as "point mutations" involving lethal or other phaenotypic effects are included in the count of lethals in the X, it is seen that the frequency of occurrence of induced translocations is probably at least as high as, or higher than, that of induced gene mutations of a detectable kind.

d. This finding requires reconciliation with the apparent rarity of "natural" translocations, as compared with "natural" gene mutations.

The explanation may partly lie in an especial liability to chromatin displacements on the part of spermatozoa, which were the type of cells chosen for treatment. But it is also probable that the natural causes of mutations differ from X-rays in that they cause a much higher frequency of gene mutations, as compared with chromatin rearrangements, than do X-rays.

3. In the second experiment reported it was demonstrated that the translocations were not previously present in the stock, but arose in the cells of the treated generation.

4 a. A fragment broken off of either of the long autosomes (chromosomes II or III) can become attached to any of the other chromosomes present, namely, the other long autosome, the X, the Y, or the small fourth chromosome.

b. The relative frequencies of occurrence of the above various kinds of attachment, as found in the present experiments, formed the following ratios, respectively: (to II or III) 1: (to X) 0.45: (to Y) 0.22: (to IV) 0.15.

c. Among these differing frequencies, we may at least attach significance to the greater value of the first frequency (representing attachments to the other long autosome) as compared with each of the other frequencies (representing attachments to the smaller chromosomes).

d. The reason for this greater tendency for attachment to a long autosome is still undetermined, though possible explanations are suggested. Thus it may be that a breakage occurring in one chromosome favors the attachment to it of a fragment from another chromosome, and that breakage is more likely to occur in a longer chromosome. Breakage of a recipient chromosome is, however, not *necessary* for the occurrence of attachment.

5 a. Breakages of and translocations from the X chromosome have been found to occur in other work of the authors, although the technique of the present experiments largely prevented their detection here.

b. Breakages and translocations of part of the Y to other chromosomes seem, according to some tests herein reported, to be less frequent than those of the long autosomes (none having been found in a rather limited series of tests).

6 a. It has been found that a large proportion of the induced translocations produce lethal effects on zygotes which receive them in a homozygous condition. Those which are not lethal commonly produce sterility and other somatic manifestations, when homozygous.

b. Three possible reasons for such effects have been discussed—simultaneous mutation at a separated locus, possible effect on gene functioning of a

change in its gene associates, and mutation or loss of genes at or adjoining the points of breakage (or attachment). At present the last hypothesis seems the most probable.

c. These lethal and other deleterious effects of most translocations, when homozygous, do not rule out translocations as factors in evolutionary change any more than the lethal or deleterious character of the vast majority of detectable gene mutations rules out gene mutations as the main building blocks of evolution. It is not to be expected that the majority of any changes occurring at random will have survival value.

TABLE 3
Results from individual P_1 males in portion of second experiment.

A	B	C	D	E	F
DESIGNATION OF P_1 MALE	MARKER CARRIED BY P_1 MALES	NUMBER OF FERTILE P_2 - P_3 CULTURES FROM "A", CARRYING MARKER INDICATED IN "B".	NUMBER OF CULTURES IN "C" CARRYING TRANSLOCATIONS	TYPE OF TRANS- LOCATION	CASE NUMBER OF TRANSLOCATION, AS REFERRED TO IN TABLE 2
1	S	5			
2	S	3	1	II-III	25
3	S	2			
4	S	12	1	II-III	26
5	S	7	1	II-III	27
8	S	2			
9	S	3			
10	S	6	1	II-III	28
11	S	5	2	{ II-III Y-III	31 34
12	S	3			
13	S	2			
15	S	1			
16	S	6	1	II-III	29
17	{ S C_v	6 3	1 1	II-III II-III	30 32
18	{ C_v S	3 3	1	II-III	33
19	{ S C_v	2 6*			
20	{ S C_v	2 2	1 1	Y-III Y-III	35 36†
Total		84	12	9II-III; 3Y-III	

* Dominant minute bristles, completely linked with C_v , appeared in 1 culture.

† Different translocation from number 35.

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STUDIES ON INHERITANCE IN PIGEONS. VII. INHERITANCE OF RED AND BLACK COLOR PATTERNS IN PIGEONS¹

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INTRODUCTION

The hereditary relations existing among the various self colors of pigeons (*Columba livia*) have been worked out by COLE and his co-workers (COLE 1911, 1912, 1914, COLE and KELLEY 1919) and by CHRISTIE and WRIEDT (1923). There are numerous color patterns in pigeons, almost each variety and sub-variety presenting a pattern somewhat different from that of any other. Some of these patterns, particularly those due to the dominant gene *A*, were studied in some of the papers mentioned above by COLE and his students. The epistatic series of black, black blue-tail, check, sooty, blue barred and blue barless was worked out by JONES (1922) and studied later by BOL (1926). Various color patterns involving mixtures of red and black have been reported on by METZELAAR (1926, 1928). STAPLES-BROWNE (1908) described "kitiness" as early as 1906, but he used

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the term to designate the "buffy edgings" which sometimes occur on black feathers.

The experiments reported on in this paper, which were begun in 1925, deal chiefly with the inheritance of two color patterns in pigeons in which there are mixtures of red and black pigment.

Facilities were adequate at all times so that each mating was made in an individual mating cage. Thus the matings were controlled and there can be no question as to the parentage of the birds produced as there might have been had the matings been made with several pairs in a large fly.

Some of the matings considered were made at the UNIVERSITY OF WISCONSIN. Several of the original pigeons used in the matings made in Texas were obtained from the UNIVERSITY OF WISCONSIN and hence had Wisconsin numbers. The Texas number and the Wisconsin numbers can be readily distinguished by the fact that a Texas number is preceded by a T, thus: T27A, whereas no letter precedes a Wisconsin number. All the progeny from a single mating are given the same number followed by different letters. Thus the progeny from mating No. T92 are given the numbers T92A, T92B, T92C, etc.

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DESCRIPTION OF PATTERNS

The inheritance of each of two different patterns showing red and black was studied. The first of these patterns shows an intimate mixture of red and black pigment in all or in a part of the feathers. The best way to describe it is that there appears to be a sprinkling of both colors over the entire feather. This color pattern is found in the Brander, the Almond Tumbler, and the Bronze Tippler. LYELL (1887) describes the Brander as follows: "The Brander is a Copenhagen Tumbler, of a black ground color, but strongly glossed with reddish bronze. A good bird should appear all over of the same color as that on the breast of the bronzed Archangel pigeon, except that the points of the primary and secondary flights, as well as a bar across the end of the tail, should be black. The Brander is smooth-headed and legged, and is, doubtless, the breed referred to by NEUMEISTER as the Fire Pigeon."

Pigeon fanciers have always been troubled with the occurrence of blacks and blues which show a bronze luster, or a red or fiery glow over and through the ground color. This condition is called kitiness, probably on

account of the similarity in appearance of kitey pigeons with the European kite (*Milvus ictinus*). Kitiness appears most commonly in the primary wing feathers. It may, however, be found over the entire bird in which case a pigeon similar to one of the breeds mentioned above is produced.

COLE (1914) in studying the genetic relationship of the self colors black and red observed that sometimes the black pigeons resulting from such crosses had more or less kitiness.

It has not been possible to photograph this type of pigeon in such a manner as to show the pattern. The flecks of the two colors are very small. Each barb of the feather may have both colors. In fact, both red and black pigment granules sometimes occur in the same barbule and sometimes even in the same cell within a barbule. Figures 1 and 2 are



FIGURE 1.—Barbules on one side of a barb, showing black with kitiness. (X360. The red, light stippling, and black, heavy stippling, pigments are mixed at and near the border lines between the red and the black areas. This is not apparent because of the use of an uncolored plate. Camera lucida drawing from feathers of T58B, black with kitiness.)

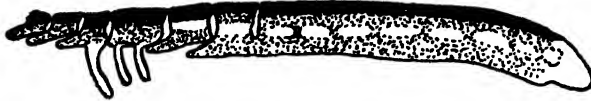


FIGURE 2.—Hooked barbule containing both pigments. (X660. Heavy stippling indicates black pigment; light stippling indicates red pigment. Camera lucida drawing from feathers of T58B, black with kitiness.)

camera lucida drawings of a portion of a feather from a pigeon of this type showing an intimate mixture of the two colors of pigments.

As will be shown below, the pattern in the different breeds of which it is characteristic is due to the same gene as that which produces kitiness in other breeds. Hence, these are all grouped together and will be referred to in this paper under the term kitiness.

The other red and black pattern studied is a very definite pattern. It is found in the Archangel pigeon. Certain parts of the body show a definite red, or, as the fancy calls it, bronze, color. These parts are the head, neck, breast, thighs and underparts, and it extends to the elongated feathers beneath the tail. The remaining portions of the bird have black plumage. These parts are the wings, back, rump and tail. The line of demarcation between the black and the bronze areas should be very distinct. Figure 3

is a non-tinted picture of an Archangel showing the parts which are black and those which are bronze.

It is a fault that is fairly prevalent in Archangels that more or less black in flecks or small areas appears on the surface of the bronze. The under-fluff in this region is black. The black sometimes appears in considerable quantities on the head and upper part of the neck, thus destroying the

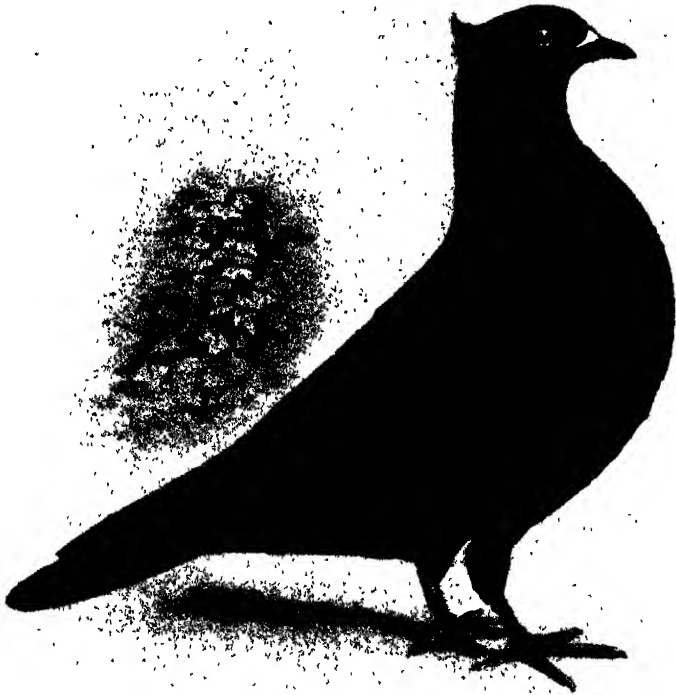


FIGURE 3.—The Archangel. The lighter areas are black; the darker areas bronze.

uniform bronze appearance. Sometimes in dark bronze Archangels so much black appears in these regions that they look black rather than bronze.

According to GOODALL the best Archangels have no bronze mixed in with the black. Each Archangel used or produced in these experiments, however, had some bronze flecking in the wings. Some Archangels show only a very slight bronze flecking. In these the flecking is in the inner vanes of the primaries and is not visible when the wings are folded. Others show more flecking in various amounts. In some there is sufficient bronze in

the wings to give them a decided reddish cast. FULTON(1895) states that there should be some bronze in the inner vanes of the primaries.

There are two types of Archangels, known as the dark bronze and the light bronze. These differ with regard to the shade of the bronze. The bronze on the dark bronze Archangel has a rich copper color. That on the light bronze is golden yellow in color.

Another characteristic of the Archangel, the inheritance of which was studied in the experiments reported in this paper, is the crest. This has nothing to do with the color pattern. The crest consists of a tuft of feathers at the back of the head compactly drawn together and culminating in a needle-like point. It should point upwards and not backwards. It varies somewhat in different pigeons with regard to its compactness and shape. An idea of the crest can be gained from figure 3.

The Archangel is known in most countries as the bullfinch pigeon. The dark bronze is known in Germany as the Kupfergimpel and the light bronze as the Goldgimpel.

EXPERIMENTAL RESULTS

Studies concerning kiteness

Relationship to black

Black and kitey birds were mated in matings numbers T1, T2, T3, T5, and T6. The results of these matings are shown in table 1.

TABLE 1
P₁ cross Black × Kitey.

MATING NUMBER	KITEY	BLACK
T1	3	0
T5	6	0
Total—T1 and T5	9	0
T2	4	2
T3	3	3
T6	1	2
Total—T2, T3 and T6	8	7
All matings	17	7

The matings are divided into two groups, those that produced only kitey offspring and those that produced both black and kitey offspring. The kitey pigeons used in these matings differed in their genetic make-up. Those which produced only kitey progeny were homozygous for gene *K* which produces kitiness, whereas those which produced both black and kitey progeny were heterozygous for this gene *K*.

Various matings were made between F_1 's from the above matings. Also backcrosses were made to both parental types. In mating T92 a black F_1 , T3C, was backcrossed to a black, 2014C. Six young were raised from this mating, of which five were black and one was dun. No kitiness segregated out.

Two black F_1 's, T3A and T3F, were mated in mating No. T47. Nine young were raised from this mating, of which six were black and three were red. No kitiness segregated out. The production of the recessive red progeny shows that both T3A and T3F were heterozygous for gene *E*, the gene for the extension of black pigment.

Kitey F_1 's were backcrossed to blacks in matings T59, T62, and T63, with the results shown in table 2.

TABLE 2
P₁ Kitey × Black. F₁ Kitey backcrossed to black.

MATING NUMBERS	KITEY	BLACK	RED
T59	1	3	0
T62	2	1	1
T63	3	3	0
Observed	6	7	
Calculated 1:1	6.5	6.5	

Deviation 0.5 ± 1.2141

Assuming that kitiness is a simple monohybrid dominant to black, a ratio of one kitey to one black is expected. It will be shown later that it cannot be determined without the proper breeding test whether or not a recessive red pigeon carries the gene *K*. Hence, in classifying the progeny from the matings given in table 2 for gene *K* the red bird is not classed in either group. The results of this backcross are very close to a 1:1 ratio.

Several matings were made between black and kitey F_1 's, with the results shown in table 3.

TABLE 3
P₁ Kitey × Black. F₁ Kitey × F₁ Black.

MATING NUMBER	KITEY	BLACK
T48	1	5
T60	2	2
T61	2	2
T90	2	7
Observed	7	16
Calculated 1:1	11.5	11.5

Deviation 4.5 ± 1.6188 .

This cross is the same as a backcross and a 1:1 ratio is expected from it. There was a marked deviation from this ratio, a deviation which is almost three times its probable error.

All the matings of kitey pigeons heterozygous for gene *K* with blacks have been given above, but in three separate tables. In order that they may all be gotten together and the results summarized, they are presented again in table 4.

TABLE 4
All matings of Kk pigeons with blacks.

MATING NUMBER	KITEY	BLACK	RED
T2	4	2	0
T3	3	3	0
T6	1	2	0
T59	1	3	0
T62	2	1	1
T63	3	3	0
T48	1	5	0
T60	2	2	0
T61	2	2	0
T90	2	7	0
Observed	21	30	1
Calculated	25.5	25.5	

Deviation 4.5 ± 2.4084

The agreement of the results from all these matings with a 1:1 ratio is fairly close, the deviation being less than twice its probable error.

Three matings were made of kitey F_1 's *inter se* with results as shown in table 5.

TABLE 5
P₁ Kitey × Black. F₁ Kitey × F₁ Kitey.

MATING NUMBER	KITEY	BLACK	RED
T46	8	5	3
T50	7	0	0
T57	1	0	0
Observed	16	5	3
Calculated 3:1	15.75	5.25	

Deviation 0.25 ± 1.3381

In this case two heterozygous, *Kk*, pigeons were mated together. On a monohybrid basis a 3:1 ratio was expected. The agreement is very close.

In order to determine whether or not the kitiness which occurs occasionally in black and blue pigeons is inherited in the same manner as the kiti-

ness studied above, the breeding records kept by COLE when he was studying the relationship of black and red were searched and a study made of the inheritance of kitiness in these pigeons. The skins, wings, or feathers of a great many of these birds have been saved. These were examined whenever possible as a check on the written description. In several instances it was doubtful from the description whether or not the bird in question was kity. If no parts had been saved it was not possible to check up on the written description. In case the kitiness should prove to be due to the gene *K*, it should be inherited in exactly the same manner as it has been demonstrated that the kitiness studied above is inherited. Tables 6, 7 and 8 are taken from Doctor COLE's records.

In the matings given in table 6 each parent was black with kitiness.

TABLE 6
Black, with kitiness × *Black, with kitiness.*

MALE PARENT	FEMALE PARENT	KITY	NOT KITY
194B	559A	4	0
972A	559A	2	2
		—	—
	Totals	6	2

In presenting the data which were taken from Doctor COLE's records, the band numbers of the two parents are given rather than the mating number. The reason for this is that in his earlier records he gave a separate mating number to each clutch of eggs. Hence, each pair of pigeons was given as many mating numbers as the number of clutches of eggs that it produced.

If this kitiness is due to a single dominant gene the expectation from matings such as those given in table 8 is either that all the offspring will be kity or that they will be produced in the ratio of 3 kity to 1 non-kity. The agreement with these expectations in table 6 is close.

Table 7 gives the results from matings in which a black pigeon with kitiness was mated with a black pigeon without kitiness.

TABLE 7
Black, with kitiness × *Black, no kitiness.*

KITY PARENT	NON-KITY PARENT	KITY	NOT-KITY
714, female	28B, male	7	7
702A, male	844A, female	0	2
		—	—
	Totals	7	9

Evidently both of the kitey parents shown in table 9 were heterozygous. These matings, then, are backcrosses of the heterozygote on the recessive type from which a 1:1 ratio is expected. The ratio secured, 7:9, deviated only one from the expected, 8:8.

Matings between black pigeons with no kitiness are given in table 8.

TABLE 8
Black, no kitiness × *Black, no kitiness.*

MALE PARENT	FEMALE PARENT	KITY	NOT-KITY
45B	56A	1	18
8A	35A	0	11
8A	7A	0	3
8A	411A	0	5
157A	411A	0	8
969A	411A	0	2
999A (Blue)	7A	0	10
888A (dun)	976B	0	11
966A (dun)	884A	0	1
1093A	1093B (dun)	0	5
1484C (blue)	1093B (dun)	0	3
46A	46B	1	4
6A	7A	0	15
493B	454B	0	14
448A	432B	0	7
862A	862B	1	0
	Totals	3	117

If black with no kitiness is the recessive condition, all of the progeny from the matings given in table 8 should be black without kitiness. The numbers here are comparatively large. One hundred and seventeen of the progeny from these matings were black with no kitiness. But three were kitey. These are exceptions to the hypothesis which has been given to explain the inheritance of kitiness.

It is said by some pigeon fanciers that once a pair of pigeons are mated they will remain mated for life, and furthermore, that they may be placed in a large fly with other pigeons but will never mate with them. Working with this idea in mind, Doctor COLE made all his earlier matings in large flies. From time to time results appeared which were not in accordance with the expectation. It seemed probable that this was due to the fact that the matings were not strictly controlled. A pigeon is, perhaps, not as true to its mate as some fanciers believe it to be. If there is an inequality of the sexes in a fly, which may result from the death of one member of

a pair, or for some other reason, the odd bird or birds are very likely to mate with some of the birds which are otherwise mated. Also, a hen occasionally lays in the wrong nest, resulting in the egg being credited to the wrong parents. As a result of the general unsatisfactoriness of this system, it was abandoned and the present system of using individual mating pens was adopted. Regarding this change, COLE and KELLEY (1919) say: " . . . these early results were obtained at a time when the matings were not controlled as closely as they have been in the later years of the work. Until 1911 the mated pairs were not isolated but were kept together in large pens, though special care was taken to prevent crossmating and to detect it if it occurred. Beginning in 1912, the use of separate pens for pedigreed matings was begun, and this practise was extended so that there were very few non-isolated pairs in 1914 and all matings have been strictly controlled since that time."

It seems plausible, therefore, to explain the occurrence of the three kitey birds noted in table 8 as being due to unavoidable errors in mating, resulting from the system then in use. The preponderance of evidence from Doctor COLE's records is that two non-kitey black pigeons when mated together do not produce kitey offspring.

The results of the matings given above prove that kitiness is dominant to self black in pigeons and that this pattern is produced by the action of a single gene which has been given the symbol *K*.

Relationship to recessive red

It has been shown by COLE (1914) that black in pigeons is a simple Mendelian dominant to red, the difference being due to the gene *E* which is responsible for the extension of black pigment over the entire bird. In the recessive condition, *ee*, the black is not extended and the red color has an opportunity to express itself. Knowing this fact, and also that kitiness is dominant to black, it was to be expected that kitiness would prove to be dominant to red. Several matings of red with kitey birds were made with the results shown in table 9.

The possibility that self red, self black, and kitiness form a series of multiple allelomorphs similar to that in guinea pigs (IBSEN 1919) has been suggested. These results disprove this theory. If these did constitute such a series, the order, on the basis of the previously known relationship of self black and self red and of the relationship established above between self black and kitiness would have to be kitiness at the top, or the dominant member of the series, self red at the bottom, or the recessive member of the series, and self black in the middle dominant to self red and recessive

to kitiness. A kitey bird which was heterozygous could then carry either the gene for self black or the gene for self red in a recessive condition, but not both. A recessive red bird could carry neither the gene for self black nor the gene for kitiness because either one, if present, would be expressed in the phenotype of the bird and it would not be red. A mating of a kitey bird which was heterozygous with a self red bird could then produce either some self red or some self black offspring, but not both, the type produced depending on which of the two lower members of the series was in the heterozygous bird. Three of the matings given in table 9, T49, T82, and T86, did not produce both self black and self red offspring, thus indicating that the theory of multiple allelomorphs does not offer the correct interpretation of the situation. Further evidence that this theory does not offer the correct interpretation is given below when it is shown that a self red pigeon can carry the gene which produces kitiness.

TABLE 9
Red × *Kitey*.

MATING NUMBER	KITEY	BLACK	RED
T49	0	2	3
T73	0	0	2
T82	1	1	4
T83	1	0	3
T86	2	2	2
T87	3	1	0
T89	0	0	4
T116	2	0	0
Observed	9	6	18
Calculated 1:1:2	8.25	8.25	16.5

$$P=0.6781$$

The results given in table 9 can be interpreted on the following basis, which seems to be the correct one. Black is a simple dominant to red, the difference being due to the gene *E*. Kitiness is a simple dominant of self black, the differences being due to the gene *K*. The gene *K* can produce kitiness only when *E* is present. It is a pattern gene affecting black. A self red bird can carry the gene *K* but it will not be expressed because there is no black on which it can be expressed. Matings of kitey birds homozygous for both *E* and *K* with double recessive self red birds should produce only kitey offspring, thus: $EEKK \times eek k \rightarrow EeKk$. If, however, the kitey birds used are heterozygous for both genes, and previous results from the mating of kitey with self black showed that the birds used in these experi-

ments were heterozygous in some instances at least, the three types would be expected in the ratio of 1 red and black: 1 black: 2 red, thus:

$$\begin{array}{c}
 EeKk \times eek \\
 \downarrow \\
 1 EeKk \text{ kitey} \\
 1 Eekk \text{ black} \\
 1 eeKk \text{ red} \\
 1 eekk \text{ red}
 \end{array}$$

This interpretation fits the observed results very closely as shown in table 9.

According to the above theory one-half of the self red birds resulting from the matings in table 9 should be heterozygous for the gene *K*, *Kk*, and the other half should be recessive, *kk*. The test for the gene *K* in a self red bird is to mate it with a self black. Any kitey progeny resulting from such a mating is due to the gene *E* from the black parent and the gene *K* from the red parent getting into the same individual.

$$\begin{array}{ccc}
 \text{Red parent} & & \text{Black parent} \\
 eeKk & \times & EEkk \\
 & \downarrow & \\
 1 EeKk \text{ kitey} \\
 1 Eekk \text{ self black}
 \end{array}$$

The appearance of only one kitey pigeon among the progeny from such a mating is definite proof that the red bird carries the gene *K*. In case the red parent is recessive for *k* only black progeny will result:

$$eekk \times EEkk \rightarrow Eekk \text{ black.}$$

In either case, of course, some self red progeny might be produced if the black bird used for testing happened to be heterozygous for *E*.

TABLE 10
Matings to test out recessive red birds for the gene *K*.

BIRD TESTED	MATING NUMBER	KITEY	BLACK	RED
T86E	T100	1	1	2
T73B	T101	3	1	0
T73A	T102	2	3	0
T49A	T103	0	2	0
T83C	T104	0	3	0
T49D	T105	1	0	0
T89B	T106	0	3	1
T82E	T107	0	2	0
T49J	T108	0	0	1
T82F	T114	1	1	0

Ten of the self red birds resulting from the matings in table 9 were mated with self black birds to test them for *K*. The results are shown in table 10.

Definite proof is given in table 10 that of the ten red birds tested five, T86E, T73A, T73B, T49D and T82F, carried the gene *K*, in four cases, at least, in the heterozygous condition. The numbers are small, but the indications are that four of the other reds tested, T49A, T83C, T89B and T82E were recessive, *kk*. From the other bird tested, T49J, only one offspring was secured and it was red, thus giving no possibility of determining phenotypically whether or not it carried *K*. The principal thing to be demonstrated was that self red pigeons can carry the gene *K*, and this was done. This substantiates the statement made above that *K* is a pattern gene and can be expressed only when black pigment is in the extended form.

Yellow and dun

In 1925 two birds were raised which showed an intimate mixture of yellow and dun in their feathers. These were T1D and an unbanded bird which was given the number T33A. Since dun is dilute black and yellow is dilute red (COLE 1914), it seemed probable that yellow and dun is the dilute form of kitiness. The gene *I* is sex-linked. Hence, any dilute (*i*) birds appearing from the mating of two intense pigeons will be females, since the female is the heterogametic sex in pigeons. Both T1D and T33A were females.

Matings were designed to test out the theory that yellow and dun is a dilute form of kitiness. Unfortunately, T1D died before any progeny were secured from her. But proof was obtained from T33A. In 1927 she was mated with a blue-barred male, T36A, in mating No. T75. Blue is a color of the intense series. From this mating there appeared among others a squab which was silver and a male. Silver is a dilute blue. From the known facts concerning the inheritance of the *I* gene (COLE 1912) it is evident that the blue male parent of this silver male was heterozygous for intensity, *Ii*, and that the yellow and dun female parent was a dilute. No dilute male would be produced if the female parent carried the dominant *I*, because each of her male offspring would receive this *I*. Any of her offspring not receiving it would be female.

The following year, 1928, T33A was mated with T3A, a self black, in mating No. T110. The object was to get the gene which was producing the mixture of yellow and dun into combination with *I* and *E*. If it were the gene *K* that was responsible for this yellow and dun condition, the combination of *K*, *I* and *E* should produce an intimate mixture of red and black.

Four offspring were obtained from mating No. T110, of which three were black and one was an intimate mixture of red and black. The appearance of this one red and black offspring from this mating is proof that T33A does carry the gene *K*.

Definite evidence was, therefore, obtained that T33A which shows an intimate mixture of yellow and dun is a dilute (*i*) and that she carries the gene *K*. The intimate mixture of yellow and dun type of coloration is the dilute form of kitiness.

Red and blue

Blue is the color which results in pigeons when black pigment is clumped (LLOYD—JONES 1915). The clumping gene (*s*) has no effect on red pigment. A blue pigeon is *EI**ss*. The gene *K* acting on an otherwise blue pigeon should produce a blue with kitiness. 2091A appeared to be such a bird, although rather dark. Mated with blue barred mates, which are definitely clumped (*s*), she produced the following progeny: 2 black check, 1 blue black barred, 1 blue with red wing bars, and 2 showing red and blue like 2091A. One of the red and blue sons, T6B, mated with a blue barred produced 1 black check, 3 blue barred, and 3 red and blue like T6B and 2091A.

If all the pigeons used in these matings were clumped (*s*), no black check progeny would be produced since black check is epistatic to blue (JONES 1922). This pattern must have come from the red and blue parent in each case. Microscopical examination of feathers from 2091A and T6B reveals the fact that some of the black pigment is clumped and some spread throughout the greater part of the cell. There is a great deal more pigment in these feathers than there is in a blue feather from a blue black barred pigeon. Hence, both 2091A and T6B belong higher in the epistatic series than blue and are probably the result of the action of gene *K* on black check.

The exact effect of *K* on a pigeon in which the pigment is completely clumped is not known at present. The one mentioned above, which is blue with red wing bars, may be the result of this combination. A definite statement on this must await future analysis.

It seems probable, from the evidence given above, that *K* is a pattern gene which, in the dominant form, acts on black pigment whether this be spread or clumped, intense or dilute.

The following chart shows the results of the genes *E*, *I*, *S* and *K* in various combinations:

E	I	$\left\{ \begin{array}{l} S \\ s \end{array} \right.$	$\left\{ \begin{array}{l} K \text{ red and black} \\ k \text{ black} \end{array} \right.$
	i	$\left\{ \begin{array}{l} S \\ s \end{array} \right.$	$\left\{ \begin{array}{l} K \text{ red and blue} \\ k \text{ blue} \end{array} \right.$
e	I	$\left\{ \begin{array}{l} S \\ s \end{array} \right.$	$\left\{ \begin{array}{l} K \text{ yellow and dun} \\ k \text{ dun} \end{array} \right.$
	i	$\left\{ \begin{array}{l} S \\ s \end{array} \right.$	$\left\{ \begin{array}{l} K \text{ yellow and silver (?) } \\ k \text{ silver} \end{array} \right.$
e	I	$\left\{ \begin{array}{l} S \\ s \end{array} \right.$	$\left\{ \begin{array}{l} K \\ k \end{array} \right\} \text{recessive red}$
	i	$\left\{ \begin{array}{l} S \\ s \end{array} \right.$	$\left\{ \begin{array}{l} K \\ k \end{array} \right\} \text{recessive yellow}$

No combination $EisK$ has been obtained. Hence, it is not known definitely that yellow and silver would result from this combination. But that would probably be the phenotype in case this combination of genes were obtained.

In the classifications used earlier in this paper where the relationship between black and kitiness was being studied, red and black, red and blue, and yellow and dun pigeons were all classified as kitey, since it is now evident that each of these types carries the dominant gene K . Likewise, black, blue, and dun pigeons were all classified as black, since each of these is recessive, kk .

Effect of A and K in combination

Another pattern gene, A , in pigeons has also been shown (COLE and KELLEY 1919) to produce its effect only when in a bird which has the extension of black pigment, that is, an E bird. Thus, A and K require identical situations as working bases. The question arises, what type of color pattern will be exhibited by a pigeon which carries E , A and K all in the dominant condition? In mating No. T76 a dominant red (A) male, T25A, was mated with a kitey female, 2091A. Two progeny resulted, one of which, T76A, was black with kitiness, the other recessive red. The recessive red bird tells nothing concerning the point in question because it is a non-extended (e) bird. A previous mating had demonstrated that T25A was heterozygous (Aa). It was, therefore, necessary to apply the breeding test to the kitey progeny, T76A, to determine whether or not she carried A as well as K . Mated with a blue male she has produced two offspring each of which was black with kitiness. While this does not constitute definite proof that T76A did not carry gene A , it does furnish some evidence that she did not.

In mating No. T77 a kitey male, 2108A, was mated with a dominant yellow check (A) female, T28A. Two offspring, T77A and T77C were produced, which were dominant red males. They showed definitely the pattern produced by A and gave no indication of the pattern produced by K . The kitey parent, 2108A, was known from the results of a previous mating to be heterozygous (Kk). Hence, it was necessary to apply the breeding test to T77A and T77C to determine whether or not either of them carried K . T77A mated with a black female produced two offspring showing the pattern due to A and four showing kitiness, the pattern due to K , demonstrating that T77A carried both A and K . T77C has produced no kitey offspring.

The breeding facts given above, particularly those obtained from T77A, furnish good evidence that A is epistatic to K in pigeons.

Variability of the pattern

The pattern produced by K is very variable. The proportionate amounts of the red and black pigments vary so that in some birds the feathers are almost completely black with only a sprinkling of red; whereas, in others the color is largely red. There are all degrees of the mixture between these two extremes.

Furthermore, the pattern varies with regard to the portion of the pigeon on which it occurs. The following types have been observed:

1. Those which have kitiness in all their feathers.

2. Those which have kitiness in all their feathers excepting their tail feathers.

3. Those which have kitiness in all their feathers excepting those on the head and in the tail.

4. Those which have kitiness in the wing and back feathers only.

5. Those which have kitiness in the feathers on the wings and head only.

6. Those which have kitiness in the wing and tail feathers only.

7. Those which have kitiness in the wing feathers only.

8. Those which have kitiness in the primaries only.

These regions of red and black pattern production agree in location with the pterylae, or feather tracts, on the pigeon. The pterylaxis of the pigeon is shown in figure 4.

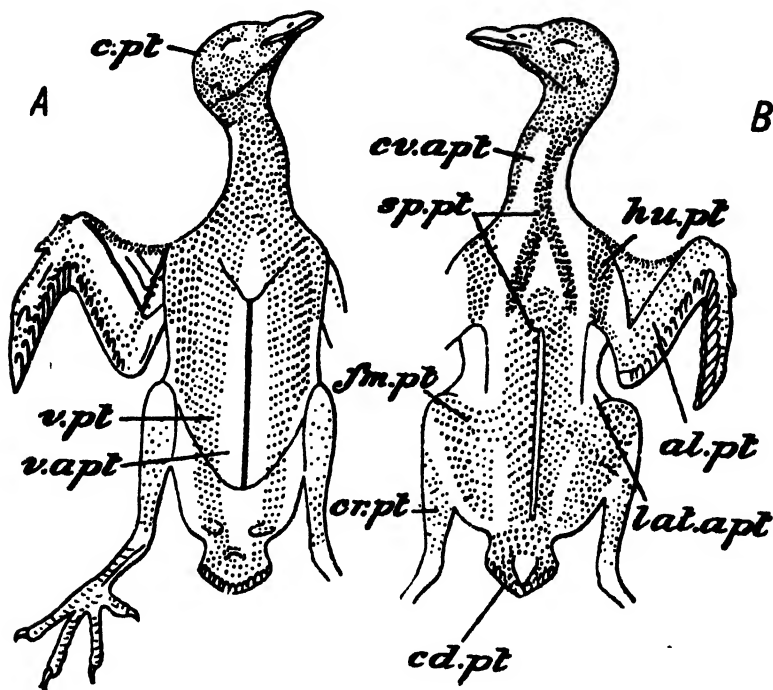


FIGURE 4.—Pterylaxis of *Columba livia*. A, ventral; B, dorsal. *al. pt.* alar pteryla or wing-tract; *c. pt.* cephalic pteryla or headtract; *cd. pt.* caudal pteryla or tail-tract; *or. pt.* ocular pteryla; *cv. apl.* cervical apterium or neck-space; *fm. pt.* femoral pteryla; *hu. pt.* humeral pteryla; *lat. apl.* lateral apterium; *sp. pt.* spinal pteryla; *v. apl.* ventral apterium; *v. pt.* ventral pteryla. (After NITZSCH.)

The production of the different types outlined above can be explained on the basis of the development of kitiness in certain pterylae and its failure to develop in others. The different types develop as follows:

1. The pattern is developed in all the pterylae, thus appearing in all the feathers on the pigeon.
2. The pattern is developed in all the pterylae except the caudal pteryla, thus appearing in all the feathers on the pigeon excepting the tail feathers.
3. The pattern is developed in all the pterylae except the cephalic pteryla and the caudal pteryla, thus appearing in all the feathers on the pigeon except those on the head and in the tail.
4. The pattern is developed in the alar, humeral and spinal pterylae, thus appearing in the feathers on the wings and back of the pigeon.
5. The pattern is developed in the alar, humeral and cephalic pterylae, thus appearing in the feathers on the wings and on the head.
6. The pattern is developed in the alar, humeral and caudal pterylae, thus appearing in the wing and tail feathers only.
7. The pattern is developed in the alar and humeral pterylae, thus appearing in the feathers on the wings only.
8. The pattern is developed in that portion of the alar pteryla which produces the primaries, thus appearing in the primaries only.

Some of the matings of kitey with kitey, and of kitey with non-kitey throw some light on the hereditary relationships of these different types of kitiness. Since the types of the parents are different in each mating, the matings are presented separately rather than in tabular form.

Mating No. T46 was between T3B, kitey throughout except the tail, and T3E, kitey throughout except the rump and tail. Fourteen progeny resulted from this mating, of which 6 were kitey throughout except the tail, 2 were kitey in the wings only, 3 were black and 3 were recessive red.

Mating No. T50 was between T2B and T2C, both of which were kitey throughout except the tail. This mating produced 4 offspring, which were kitey throughout except the tail, 2 which were kitey throughout except the tail and head, and 1 which was kitey in the wings only.

Mating No. T58 was between 2098A, kitey in the wings, remainder of feathers black, and 2110A, kitey throughout. The progeny were 3 kitey throughout, 2 kitey throughout except the tail, and 1 recessive red.

Mating No. T64 was between 2090A, kitey in the wings, remainder of feathers black, and 2112A, kitey throughout. This mating produced 1 offspring which was kitey throughout and 2 which were kitey throughout except the rump and tail.

In no case in any of the four matings given above were any progeny produced which had kitiness in the feathers in a pteryla in which the feathers were not kitey in one or both parents. In each mating, however, progeny were produced which had kitiness in the feathers from fewer

pterylae than did one or both parents. These facts indicate that the presence of a pattern in a certain pteryla is dominant to its absence from this same pteryla.

In mating T60, a black pigeon T2F was mated with T2A, kitey throughout. The progeny were 1 kitey throughout except the tail, 1 kitey in the wings and tail, black elsewhere, and 2 black.

T2F, black, was mated with T2B, kitey throughout except the tail, in mating T90. This mating produced 1 kitey on wings, back and breast, 1 kitey on wings only, and 6 sooty, black, and dun—not showing the pattern.

From each of these matings progeny were produced which had kitiness in the feathers from fewer pterylae than did the kitey parent. These results are in agreement with those noted above from matings of two pigeons, each of which was kitey.

There are three possible explanations of the genetic relationships of the different types of kitiness. (1) That there is a series of multiple allelomorphs determining the particular type of the pattern. (2) That there is one gene, *K*, which determines whether or not the pattern is produced and that the distribution of the pattern among the different pterylae is due to modifying genes. (3) That *K* is a variable gene.

In case a series of multiple allelomorphs is concerned, it would be expected, on the basis of the breeding facts presented above, that the members of the series for greater extent of the pattern would be dominant to those for lesser extent of the pattern. Then the mating of a pigeon with the pattern in the feathers from certain pterylae with a pigeon without the pattern would produce no offspring with the pattern in the feathers from any other pteryla. Disproof of this theory is given by mating T117, in which 2098A, kitey in the wings only, was mated with T87D, dun. One of the progeny, T117C, was kitey throughout except the tail. T117C thus had kitiness in the feathers from several more pterylae than did its kitey parent, 2098A.

The greater extension of the pattern in T117C than in 2098A can be explained by assuming that the extent of the pattern is determined by modifying genes and that the dun parent, T87D, transmitted to T117C modifying genes for greater extent of the pattern. None of the observed breeding facts are contrary to the hypothesis of modifying genes. It probably offers the correct explanation of the facts. There is a marked similarity in the breeding behavior of this pattern and of the hooded pattern in rats which was studied by CASTLE (1924).

However, the breeding facts are not of such a nature as to permit posi-

tive denial of the possibility that *K* is a variable gene, of the nature suggested by DEMEREC (1928), ANDERSON and EYSTER (1928), EMERSON (1917) and others. To decide whether the variability is due to modifying genes or to a variable gene it would be necessary to attempt to produce strains of pigeons which would breed true for different variations of kitiness. The best way to do this would be to inbreed (EAST and JONES 1919). If such true breeding strains could be established, it would be demonstrated that the variations in kitiness were caused by modifying genes, which had been gotten into a homozygous condition in each of the true breeding strains. If such true-breeding strains could not be established, it would then be time to look into the question of variable genes.

Studies concerning the Archangel

Relationship of the pattern to black

Archangels were mated with self blacks in matings Nos. T4 and T43. The results are presented in table 11.

TABLE 11
Archangel × *Black*.

MATING NUMBER	BLACK	BRONZE AREA	ARCHANGEL	NO CREST	CREST
T4	3	2	0	5	0
T43	2	2	0	2	0
Total	5	4	0	7	0

No progeny showing the Archangel pattern were produced, indicating that this pattern is recessive to self black. However, four of the nine offspring did show a bronze area in the breast. This is very small in the T4 birds and rather extensive in the T43 birds. This indicates that black is incompletely dominant to the Archangel pattern. None of the F_1 's showed the Archangel crest on the head. It is not always noted on the description sheet whether or not the bird in question has a crest. For that reason the total number of birds used in studying the crest does not always agree with the total number used in studying the Archangel pattern.

Wisconsin mating No. 2094 was between 2014C, a black male, and 2086A an Archangel female. Thirteen offspring were produced from this mating, of which six were self black and seven black with a small bronze area. Thus the results were similar to those secured from the crosses made in Texas. With regard to the crest, however, the situation is different. Of the 2094 birds, five are described as having the crest, three as not having it, and no mention is made of it in describing the other five. No definite explanation

can be given as to why some of the 2094 birds have the crest. But in view of results which will be presented below, 2014C must have been heterozygous for the gene *C* which, in the recessive condition (*cc*), is shown to produce the crest. The pedigree of 2014C was examined as completely as possible in the record books of the Department of Genetics of the UNIVERSITY of WISCONSIN, but no ancestors were found in his pedigree in the description of which any mention was made of a condition of the head feathers which might be taken as even a resemblance to the Archangel crest. The only suggestion was found in the description of 45A, a brother to 45B which appears in the fifth generation on the sire's side of the pedigree of 2014C and in the seventh generation on the dam's side of his pedigree. 45A is described as black with a decidedly reddish cast to the breast. This condition may be the same as the bronze area noticed in the breasts of several of the hybrids between blacks and Archangels which are reported on in this paper. It might, therefore, be taken as an indication that there is some Archangel ancestry in the pedigree of 2014C. In case that is true, the gene for the crest could have been carried along in the recessive condition, masked by its dominant allelomorph, and thus reached 2014C. Then 2014C, when mated with a recessive, was able to produce progeny showing the recessive condition—the crest. Furthermore, as pointed out in section B below, crests of feathers occur on the heads of pigeons of other breeds than the Archangel. It is possible, then, that 2014C had inherited the gene for the crest from an ancestor that was not Archangel.

Black non-crested F_1 's from the cross of black on Archangel were mated to produce F_2 's. The results obtained are given in table 12.

TABLE 12
P₁ Black × *Archangel*. *F₁ Black* × *F₁ Black*.

MATING NUMBER	BLACK	BRONZE AREA	ARCHANGEL	NO CREST	CREST
T38	8	1	0	5	3
T39	9	8	1	16	1
T67	0	1	1	0	2*
	17	10			
Observed	27		2	21	4
Calculated 15:1	27.1875		1.8125		
Calculated 3:1				18.75	6.25

* Not counted in the totals because both parents had the crest.

Deviation, Archangel pattern $0.1875 \pm .8793$

Deviation, Crest 2.25 ± 1.4604

Before discussing these results it will be well to consider also the backcross data. F_1 's from the cross of black \times Archangel were mated with pure Archangels. The results are presented in table 13.

TABLE 13
P₁ Black \times Archangel. F₁ Black backcrossed to Archangel.

MATING NUMBER	BLACK	BRONZE AREA	ARCHANGEL	NO CREST	CREST
T45	3	0	1	2	2
T72	2	0	0	1	1
T94	1	1	2	3	1
T95	2	1	1	4	0
T96	2	1	0	1	2
T97	0	3	1	1	3
T98	0	4	0	0	4
T118	0	2	0	0	2
	10	12			
Observed	22		5	12	15
Calculated 3:1	20.25		6.25		
Calculated 1:1				13.5	13.5

Deviation, Archangel pattern 1.75 ± 1.5176

Deviation, Crest 1.5 ± 1.7537

In both tables, 12 and 13, several pigeons are listed as being black with an area of bronze feathers on the breast. These descriptions are based on the juvenile plumage. The bronze area which occurred on the breasts of these F_2 and backcross pigeons was quite variable in size. In some it was very small, being much like that on the breasts of some of the F_1 's. In others it was much larger, including all of the parts which are bronze on an Archangel excepting the head and upper part of the neck, the region which is feathered from the cephalic pteryla. No attempt was made to divide the birds with the bronze areas on their breasts into different classes. In tables 12 and 13 they were all classed with the non-Archangel group.

According to both the F_2 data and the backcross data, the difference, in the juvenile plumage, between the Archangel pattern and the lack of complete development of this pattern (including both black and bronze area birds) is due to two genes, both of which must be homozygous recessive in order that the Archangel pattern may be produced. These genes have been given the symbols P and Q . Since there is 1 double recessive individual out of 16 in the F_2 of a dihybrid, the expected F_2 ratio is 15 not Archangel: 1 Archangel. On the basis of this same explanation a ratio of 3 not Archangel:1 Archangel is expected from the backcross of F_1 on Archangel.

The results obtained in both the F_2 and the backcross are very closely approximated by the results calculated on the basis of the explanation given above, as shown in tables 12 and 13. In the case of the F_2 ratio there is a slight excess of the Archangel pattern, but the deviation is much smaller than its probable error. In the backcross ratio there is an excess of blacks, but the deviation from the expected ratio is not significant, being only slightly larger than its probable error.

Very few of the F_2 and backcross birds with the bronze areas on their breasts were saved until they had passed through molt. This is an unfortunate circumstance because some of those which were saved had very good Archangel patterns in the adult plumage. It would be instructive to know what would have happened in the case of each of these birds. In the case of the backcross birds, T98E and T98D, each had a good Archangel pattern following molt. T98C, however, retained the black on the head and neck. Likewise, only three F_2 bronze area birds lived to pass through molt. These were T38A₂, T39A₂ and T39F₂. Each of these birds was described as having a small bronze area in the breast in the juvenile plumage. In the adult plumage T38A₂ has no bronze in the breast and the other two birds have still a very small amount of bronze in this region. Thus, some of the bronze area birds changed to the Archangel pattern following molt, whereas others did not.

The question arises, to what agency can this difference between juvenile and adult plumage be attributed? Two possibilities suggest themselves. The first is that of the delayed action of the gene. It is a well-known fact that horses which will be gray at maturity are black when foaled and become gray only after shedding the first coat of hair. Likewise, it is known that many tow-headed children become darker headed as they grow older.

PUNNETT (1923) shows that as regards henny-feathering in male chickens, the juvenile plumage may show an intermediate condition, whereas, after molting complete henny feathering may be shown. These intermediates, when bred from, behave as hennies and may produce sons which are almost completely henny. He says, "The facts seem to suggest that in some cases where the cock is heterozygous the inhibiting substance is not produced in sufficient bulk at a stage sufficiently early to suppress the developing cock plumage completely. Later on, as the testes increase in size, the substance is produced more freely, and almost, or quite complete hen-feathering results."

MORGAN, BRIDGES and STURTEVANT (1925) writing on the action of genes say, "Whether the genes are active all the time, or whether their

activity is correlated with certain phases of development, we do not know at present and can scarcely even surmise."

The explanation of the change in pattern on this basis is that there is one gene which accounts for the difference between Archangel pattern and not Archangel pattern in pigeons. This gene must be double recessive in order that the Archangel pattern may be produced, that is, that the feathers on the bronze parts of the Archangel may be prevented from producing black pigment. The action of these genes is delayed in certain regions, particularly in the cephalic pteryla, in some of the recessive birds, permitting the feathers in the juvenile plumage in these regions to become black. Throughout later life, however, these genes are active and the bird carrying them exhibits the Archangel pattern following molt. In order to account for the production of some birds with the Archangel pattern in their juvenile plumage in the F_2 and backcross generations, it is necessary to assume that in some cases these genes were active sufficiently early in development to produce this pattern in the juvenile plumage.

The other explanation is that there is one gene, P , which accounts for the difference between Archangel and not Archangel, and that the double recessive, pp , is modified in its action in the juvenile plumage by another gene, Q , which prevents the action of pp in the feathers from the head pteryla. But Q acts only in the juvenile plumage. It is inactive in the older pigeon. Hence, $ppQQ$ and $ppQq$ pigeons are black with a bronze area on the breast in their juvenile plumage, whereas in their adult plumage they exhibit the Archangel pattern. The expectation on this basis is that the F_2 generation would show a ratio of 15:1 in the juvenile plumage and of 3:1 in the adult plumage. The backcross ratio would be 3:1 in the juvenile plumage and 1:1 in the adult plumage.

There are no breeding facts available to aid in deciding which possibility is correct. The writer is inclined, however, to accept the latter. It seems probable that if the gene were delayed in its action it would be delayed in all cases so that on that basis no pigeons with the Archangel pattern in the juvenile plumage would be produced in the F_2 or backcross generation. But some were produced. To account for them it would be necessary to assume that the same gene is active at different stages of development in different individuals, which may be possible but is not probable. The second hypothesis, on the other hand, accounts for the production of both types in the F_2 and backcross generations and in the ratios observed.

Wisconsin mating No. 2216 was between 2087A, an Archangel male and 2094E, a dun checker female, which was an F_1 from a black \times Archangel cross. It was, therefore, a backcross similar to those given in table 13.

Two progeny resulted from this mating, both of which were black. This is not out of line with the expected ratio of 3 not Archangel:1 Archangel.

Mating No. T54 was between 2094C, an F_1 from a black \times Archangel cross, and 2014C, a self black. This mating was a backcross of the F_1 on black. Seven offspring resulted from this mating, all of which were self black as was to be expected on the basis of the explanation offered above.

The most probable explanation of the occurrence of a black with a small area of bronze on the breast is that P is incompletely dominant. The other suggestion was offered that there is a gene for the production of the bronze area. If this were true, either one type or the other, the self black or the black with bronze area should breed true. But each type has produced the other. Mating No. T38 was between two self black F_1 's. This mating produced eight self black and one black with bronze area. Mating No. T39 was between two F_1 's, each of which was black with a bronze area. The progeny were nine self black and eight black with bronze area.

BESSMERTNAYA (1928) has reported a dominant gene for the Archangel pattern. METZELAAR (1926) also states that the Archangel pattern is of a dominant type. But, as noted in this paper, the results obtained both in Wisconsin and in Texas are not in accord with this theory. The data available demonstrate, without exception, that the Archangel pattern is recessive.

No attempt has been made thus far to combine the Archangel pattern with clumped black pigment or with dilute black pigment. LAVALLE and LIETZE (1905) and METZELAAR (1926) describe Archangels that are blue in the regions which are usually black. It would seem that these are Archangels with clumped pigment, ss . No descriptions have been seen of Archangels with dun or silver pigment, but it seems probable that such types could be produced by the proper recombinations of the genes. LAVALLE and LIETZE also describe Archangels which are white in the regions which are usually black.

Inheritance of the crest

The data presented in tables 11, 12 and 13 show that the Archangel crest is inherited as a simple Mendelian recessive to the usual smooth feathered condition on the head. The gene for this difference is given the symbol C . A pigeon without the crest is either CC or Cc . One which has the crest is cc . The agreement of the observed and calculated ratios in both the F_2 and the backcross generations is good. In the F_2 there is an excess of birds without the crest, but in the backcross the excess over the calculated number is among the birds which have the crest. The deviations, small though they are, thus tend to offset each other.

These results are comparable with those of CHRISTIE and WRIEDT (1923) who studied the inheritance of the Rundkappe (round cap) in the Norwegian Petent pigeon. In the Rundkappe of this breed the feathers on the back of the head from ear to ear are reversed and form a sort of cap or hood. The Rundkappe was found to be due to one recessive gene which they call r_u . Since the behavior of r_u is the same as that of c it is probable that the two symbols represent the same gene. STAPLES-BROWNE (1906) reported that the shell of the Nun pigeon is inherited as a simple Mendelian recessive to the smooth headed condition. This shell is similar to the Rundkappe, the feathers from ear to ear being reversed, forming a cap or hood. The crests on the heads of some of the segregates from Archangel crosses are wider in extent than those on typical Archangels. These crests in some cases reach from ear to ear. A crest of feathers on the head is typical for a Red Crested Helmet pigeon. A pigeon reputed to be a Red Carneau, shown in the pigeon show at Brenham, Texas, in February, 1929, which was judged by the writer, had a crest of feathers on its head which resembled very closely the crest on the head of an Archangel. It may be that all head caps or crests on pigeons are due to the same gene, and that the particular shape which the crest assumes is determined by modifying genes.

Relationship of the pattern to recessive red

The relation of the Archangel pattern to recessive red has been studied, but very unsatisfactorily. For some reason or other, matings made between these two types have not done well and very few offspring have been produced. Only two matings have produced any at all. The results of these are given in table 14.

TABLE 14
Archangel × *Recessive Red*.

MATING NO.	BLACK	ARCHANGEL
T 88	3	1
T111	2	0

Theoretically all of the progeny from these matings should be black if the parents were homozygous. The Archangel parent furnishes the gene *E* for the extension of black pigment and the red parent furnishes the genes *P* and *Q* which make self color dominant over the Archangel pattern. The Archangel produced in T88 can be accounted for by assuming that the red parent, 1875C, was heterozygous for *P* and *Q*.

All of the birds produced from these two matings with the possible

exception of T111G were kitey. This kitiness could have come from the red parent. It is known from the results of another mating that 1875C, the red used in T88, was heterozygous for *K*, the gene which produces kitiness. As stated above in the description of the Archangel, a great many pigeons of this breed have red flecking in their wings. It has been determined that this red flecking is dominant to self black. It is inherited in the same manner as the kitiness described earlier in this paper, and undoubtedly is identical with it. The Archangel used in each of the matings under consideration had kitiness in the wings. Hence, the kitiness in the progeny was inherited from the Archangel parent.

The Archangel Fancy reports the occasional production of an "all bronze" Archangel. This is undoubtedly due to the segregation of a recessive. Whether or not this "all bronze" Archangel is a recessive red cannot be stated because none of the "all bronze" Archangels have been bred, or, even observed, by the writer of this paper. It would seem, however, to be a good suggestion for a working hypothesis that the production of these "all bronze" Archangels is due to the segregation of *ee* birds.

Effect of *A* in combination with the Archangel pattern

Two matings were made between Archangels and pigeons carrying the gene *A*. In one of these, T44, a yellow bar (*A*) male, T29A, was mated with an Archangel female, T16A. Five progeny were produced, two dominant reds and three dominant yellows. These all show very definitely the pattern due to *A* and no sign of the Archangel pattern. This is as was to be expected because *A* is dominant to self black, whereas the Archangel pattern is recessive to self black.

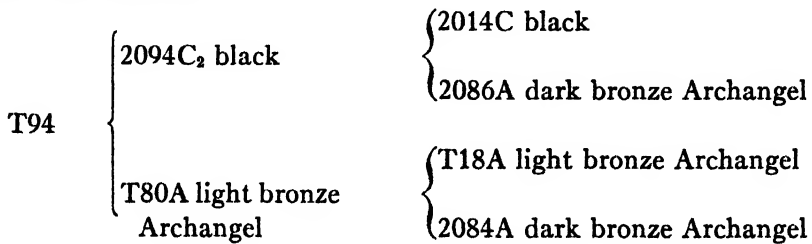
Mating No. T79 was between an Archangel male, T21A, and a dominant red female, T31A. Two offspring were produced. T79B, a male, was dominant red. T79A, a female, showed a fairly good Archangel pattern. Only the male offspring from this mating would receive the gene *A* because it is sex-linked (COLE and KELLEY 1919). These two matings demonstrate that whenever gene *A* is in combination with the Archangel pattern, the pattern due to the action of *A* is produced.

Dark bronze and light bronze

As pointed out in the description of the Archangel, there are two distinct types known as the dark bronze and the light bronze. Matings were made between birds of the two types with a view of studying the genetic relationship between them.

Mating No. T41 was between T14A, a dark bronze Archangel male, and T15A, a light bronze Archangel female. This mating produced one typical dark bronze Archangel and four birds which showed the Archangel pattern with a shade of bronze intermediate between dark bronze and light bronze, but approaching rather closely to the dark bronze.

Mating No. T80, which was between T18A, a light bronze Archangel male, and 2084A, a dark bronze Archangel female, produced one light bronze Archangel offspring. The bronze on this pigeon, T80A, is, however, darker than the shade desired in light bronze Archangels. T80A was mated in T94 with a black, 2094C₂. A two generation pedigree of the T94 birds is as follows:



Two of the progeny, T94A and T94C, were light bronze Archangels. Two others, T94G and T94H, were black with large light bronze areas on the breasts. T94B was black check with a small bronze area, the shade of which was not noted. T94D was black.

Mating No. T42 was between two dark bronze Archangels, T21A and T22A. This mating produced three dark bronze Archangels, one light bronze Archangel, and two Archangels with an intermediate shade of bronze.

These matings do not solve the problem of the relationship between the two types of bronze. No further analysis has, however, been made. The shade of bronze produced was found to be very variable. It was impossible to describe the shade of bronze definitely without a set of color standards, which were not available. Grayish areas appeared in the throats of some of the birds.

An attempt was made to classify the progeny from the above mentioned matings on the basis of their juvenile plumage. Not many of them were carried over to their second year. It has now been observed that the adult plumage assumed after molt is much more definitely either dark or light bronze than is the juvenile plumage. Further study of this problem will have to be made on the basis of adult plumage rather than of juvenile plumage.

METZELAAR (1928) has suggested that the difference between the two types of Archangels is due to a dominant dilution gene which in the homozygous dominant or heterozygous condition produces the light bronze and in the homozygous recessive condition produces the dark bronze. If this were true, crosses between the two types should produce only light bronze progeny or equal numbers of both types, depending on whether the light bronze parent were homozygous or heterozygous for the gene responsible for dilution. As noted above, mating T80 produced one light bronze offspring, T80A, which in turn produced light bronze offspring but no dark bronze in T94. But mating T41 produced five offspring, not one of which was light bronze. Granting that the light bronze parent used in T41 was heterozygous, the production of five recessives (dark bronze) and no dominants (light bronze) from such a mating would happen due to chance alone only once out of thirty-two times. Thus, the odds are seen to be against the suggestion of the dominant diluter.

If dark bronze were the recessive condition, matings between dark bronze birds should produce no light bronze offspring. However, as indicated above, such a mating, T42, did produce a light bronze offspring, a fact which is not at all in accord with the theory of a dominant dilution gene. METZELAAR had suggested previously (1926) that the dark bronze was an intense (*I*) condition, whereas the light bronze was dilute (*i*). This was easily checked up on because the relationship of intense and dilute pigeons is known definitely (COLE 1912). Intense is dominant to dilute and the gene *I* is sex-linked.

In mating No. T43 a light bronze Archangel male, T18A, was mated with a dun female, T27A. Dun is a dilute color. The result was four black offspring, two males, one female and the sex of the other is not known. Black is an intense color. These T43 birds must necessarily have received the *I* from their light bronze Archangel parent, T18A. In case T18A is homozygous for *I* nothing but intense progeny would be expected from this mating.

Mating No. T44 was the reciprocal of T43. A yellow bar (dilute) male, T29A, was mated with a light bronze Archangel female, T16A. From this mating there were secured four progeny, of which the sex has been definitely determined. These consisted of two dominant red (intense) males, and two dominant yellow (dilute) females. Here again the gene *I* must necessarily have been received from the light bronze Archangel parent. These results agree with the expectation that when a dilute male is mated with an intense female the male offspring will be intense and the female offspring dilute. Adequate proof is furnished from these two matings, T43

and T44, that the light bronze condition is not due to the sex-linked dilution gene *i*.

Black feathers in recessive reds

Very occasionally a recessive red pigeon has one or a very few black feathers. This condition has been observed in 1057A, 1875C, T49D and T55C. Since red is recessive to black this is an unusual condition. A recessive red pigeon is *ee*. A black is either *EE* or *Ee*.

Only one black feather appeared in 1875C. This was found among the coverts of the left wing, which were red. This feather had a sprinkling of red throughout, which was undoubtedly due to the gene *K*, since it has been proved, as outlined earlier in this paper that 1875C carried *K*.

Also, only one black feather appeared in T49D. This bird was recessive red but had a great deal of white on it. The one black feather appeared on the rump in the midst of an area of white feathers.

In T55C an area of fourteen black feathers occurred just under the right shoulder joint, and another area of three black feathers occurred on the front part of the humeral portion of the right wing.

An area of several, probably six or eight, black feathers, occurred on the back of 1057A. This group of feathers was completely surrounded by red feathers.

T49D and 1875C have been used in matings, but only with black mates. They have produced no recessive red progeny. Hence, no data are available to indicate whether or not the appearance of the black feathers is hereditary. This condition cannot be considered as a secondary sexual character because 1057A was a female, whereas the others were males.

A number of analogous situations have been reported in several different animals. In some cases the aberrant color appearing has been of a type that is normally dominant to the color exhibited by the remainder of the animal or organ. In other cases the aberrant color has been of a recessive type.

PINCUS (1929) reports three mice which were heterozygous for black and chocolate (*Bb*) each of which was black with a small area of chocolate in its pelage. He believes that these chocolate areas may be accounted for either by non-disjunction resulting in cells from which the chromosome carrying *B* is missing, or by a temporary reversal of dominance of such a nature that pigment deposition in these areas was governed by the *b* (chocolate) gene.

A blue area on a black rabbit was reported by CASTLE (1929). This condition was transmitted to the offspring in certain instances. The rabbits in question were heterozygous for intense and dilute, *Dd*. CASTLE

says there are three possible explanations of this blue patch: 1. A somatic mutation has occurred resulting in loss of the *D* chromosome from an area of the skin, in which *d* is left free to express itself. 2. Reversal of dominance of *D* over *d* in this particular area. These two possibilities are rejected by CASTLE. 3. The explanation to which CASTLE is inclined is that the supposed gene *D* in this case is really a mosaic and that some of the genomeres of this gene are *d*. As a result of continued somatic cell divisions, some of the daughter cells come to be *d* in all or nearly all of the genomeres of this mosaic gene and produce the dilute color, in this case blue.

PATTERSON (1928) has produced a large number of *Drosophila* after treatment with X-rays in which the eyes which would normally be red have some white ommatidia. He has explained the production of these white ommatidial areas in two ways. In some cases there has been a gene mutation from *W* (red) to *w* (white) in the somatic cells which were ancestral to these particular ommatidia. In other cases the X-ray has produced chromosome abnormalities, involving the loss of the X-chromosome, or at least that part of it which carries the dominant gene *W*.

The foregoing cases, each of which involves the production of a recessive area, are open to several explanations. Not all of the explanations will apply, however, in case the aberrant area is of a dominant type. The loss of a chromosome or a part of a chromosome would not change the type because the homologous chromosome carries the same gene as does the one that was lost. Reversal of dominance does not apply because the gene for the aberrant dominant color is not in the normal cell, hence cannot be reversed with. The genome theory might account for the facts in case a preponderance of recessive genomeres dominated a few dominant genomeres. During somatic cell divisions the relative proportions of the dominant and recessive genomeres might become changed to such a proportion that more dominant than recessive genomeres were in the gene and this gene became dominant over its recessive allelomorph. Such a change in the gene is essentially a gene mutation. The remaining explanation is that of gene mutations, which is just as applicable to the production of dominant aberrant areas as to the production of recessive mutant areas. This explanation has been offered as the most probable one for the cases given below.

PATTERSON has produced, by means of X-ray treatment, a dominant somatic mutation in *Drosophila*. This is a case in which apricot eye color has mutated to near red. It is an area on one eye consisting of about twenty-five facets.

TIMOFEEFF-RESSOVSKY (1929), also using X-ray treatment, reports the production of three dominant somatic mutations in some of the facets of the eyes of *Drosophila*. One case was from white to red, another from eosin to red, and the third from white to a light red allelomorph.

The occurrence of a black spot on a red steer has been reported by the writer (HORLACHER 1928).

WRIGHT and EATON (1926) report the occurrence of a guinea pig which had two red agouti (intense) areas on an otherwise yellow agouti (dilute) pelage. Both of his parents were dilute. He transmitted the dominant mutant gene for intensity to about one-third of his progeny. WRIGHT and EATON offer the explanation that this guinea pig was $c^d c^d$ initially but that one c^d mutated to C in a cell ancestral to part of the soma and also to part of the germinal epithelium.

The appearance of black feathers in recessive red pigeons can be explained on the same basis as the appearance of dominant aberrant areas in other animals. A recessive red pigeon is ee . A black pigeon is either EE or Ee . The explanation is that the pigeon is recessive red, ee , originally. A mutation occurs from e to E in one of the somatic cells. This cell and its descendants are Ee . Feathers developing from cells descended from this cell will be black as a result of the action of E . In case only one feather is black, as in 1875C and T49D, the mutation occurred late enough in development so that only one feather papilla received the mutant gene E . In case several feathers are black, as in 1057A and T55C, the mutation occurred in a cell which was ancestral to several feather papillae, each of which received the mutant gene E .

SUMMARY AND CONCLUSIONS

1. This paper is a study of the inheritance of two different red and black color patterns in pigeons and of their relationships to the various self colors. These patterns are:

A. Kitiness. An intimate mixture of red and black over the entire bird.

B. Archangel. The pattern found in the Archangel pigeon in which the two colors are confined to definite areas of the bird.

2. The inheritance of the crest on the Archangel was studied also.

3. Each of these patterns is produced by a pattern gene or genes which produce their effects only when they have as a basis extended black pigment (E).

4. Kitiness is produced by a dominant gene which has been given the symbol K .

5. Recessive red pigeons may carry *K*, but it is not expressed because the black pigment is not extended (*e*).

6. The effect of the dilution gene (*i*) on a pigeon which would in the intense (*I*) series show an intimate mixture of red and black is to produce an intimate mixture of yellow and dun.

7. The experimental results which are given indicate that the effect of the clumping gene (*s*) on a pigeon which would in the spread condition (*S*) show an intimate mixture of red and black is to produce an intimate mixture of red and blue.

8. Evidence is presented which indicates that the gene *A* is epistatic to the gene *K*.

9. The pattern produced by *K* is very variable.

10. The Archangel pattern is recessive to self black. It differs in expression, in regard to both the extent of the pattern and the shade of bronze, in the juvenile plumage and the adult plumage. There apparently are two genes concerned, which have been designated *P* and *Q*. *pp* produces the Archangel pattern, but *Q* prevents it from being produced on the head and neck in the juvenile plumage.

11. Matings between Archangels and pigeons carrying the dominant pattern gene *A* produce progeny which show the pattern due to *A*.

12. The difference between dark bronze and light bronze Archangels is not due to the intensity gene *I*.

13. The crest on the head of the Archangel is inherited as a simple Mendelian recessive. The gene for this has been designated as *C*.

14. Four examples are cited of recessive red pigeons which had one or more black feathers. This condition is explained as being due to a dominant somatic mutation.

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TRANSLOCATIONS INVOLVING THE THIRD AND THE FOURTH CHROMOSOMES OF *DROSOPHILA MELANOGASTER**

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INTRODUCTION

The chromosomal aberrations known in *Drosophila* as well as in other animals and in plants may be divided in two classes. The first class comprises aberrations involving whole chromosomes or groups of whole chromosomes. Polyploidy and non-disjunction are the most frequently observed phenomena of this kind. The study of the chromosomal aberrations belonging to this class has given much information concerning the role of chromosomes in general and the role of individual chromosomes in particular in the transmission of hereditary characters. The most clear and convincing proof that the chromosomes are carriers of hereditary material is that secured by the study of non-disjunction of the first and of the fourth chromosomes of *Drosophila* (BRIDGES 1916, 1921). The aberrations involving sections of chromosomes constitute the second class. Here belong translocation, duplication, inversion and deficiency of section of a chromosome (see BRIDGES 1923). These phenomena are comparatively little known, although they furnish favorable material for the investigation of the distribution of hereditary material within the chromosomes. Translocations seem to be especially valuable for the study

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of the problem just mentioned because they are most likely to produce differences in the genetical behavior of some characters together with visible alterations of the chromosomes.

The term "translocation" was proposed by BRIDGES (BRIDGES 1923, MORGAN, BRIDGES, STURTEVANT 1925) "for cases in which a section of chromosome is removed from its normal location but is present in an abnormal location." Theoretically, a section removed from its normal location in one chromosome may become attached either to a new locus in the same chromosome or to another chromosome. Both intrachromosomal and interchromosomal translocations are, therefore, possible. Most of the translocations described so far (BRIDGES 1923; MULLER and PAINTER 1929; PAINTER and MULLER 1929; also the translocations described in the present paper) are interchromosomal, but DUBININ (1929) recently found also an intrachromosomal translocation.

In the case of the "Pale" translocation described by BRIDGES (1919, 1923), a section of the second chromosome carrying the genes from plexus to speck was broken off and became permanently attached to the third chromosome near the gene ebony. Flies heterozygous for the translocation, that is, carrying one deficient second chromosome and one third chromosome with the section of the second attached to it, are nearly normal in appearance but give abnormal genetical results when crossed to flies free from the translocation. Four kinds of zygotes are produced in such a cross: (1) carrying both the deficient second chromosome and the third chromosome with the attached section, (2) the deficient second chromosome and the normal third, (3) a normal second and the third with the attached section, (4) normal second and third chromosomes. Zygotes 1 and 4, representing the parental combinations of the chromosomes, give flies heterozygous for the translocation and normal flies respectively. Zygotes 2 and 3 represent the recombinations of the parental chromosomes. Zygotes 2 die; zygotes 3 sometimes survive, but give rise to flies abnormal in appearance and weak in constitution because they carry a duplication for a section of the second chromosome. The elimination of zygotes 2 and the partial elimination of zygotes 3 result in an apparent linkage of the genes belonging to the second and the third linkage groups.

The apparent linkage observed in the case of the "Pale" translocation suggests a method of finding translocations in general. Each of the chromosomes of a fly may be marked by appropriate genes. If such a fly carries no translocation, all the possible recombinations of the "marking" genes must be present in its progeny because of the free recombination of the parental chromosomes in the gametogenesis. However, if a fly with

"marked" chromosomes carries a translocation involving any two of the chromosomes, a linkage of the genes localized in those chromosomes will be observed. Hence, some of the expected classes of the offspring will be absent, or will manifest somatic peculiarities due to the disturbance of the normal genic balance.

The "Pale" translocation arose spontaneously; the same is true in respect to a few other cases of translocations (see MORGAN, BRIDGES, STURTEVANT 1925, pp. 178-179). The frequency of the spontaneous origin of translocations seems to be, however, so low as to make an attempt to secure them at will impracticable. The production of translocations in comparatively large numbers became possible only when MULLER (1928a, 1928b) and WEINSTEIN (1928) discovered that the frequency of translocation is very considerably increased in the progeny of flies treated by X-rays.

Recently MULLER and PAINTER (1929) and PAINTER and MULLER (1929) have published a preliminary account of their cytological and genetical investigations on the translocations involving different chromosomes of *Drosophila melanogaster*. Most of their conclusions coincide with the conclusions drawn in the present paper on the basis of my own material.

The present work was done in 1928 and 1929 at the MARINE BIOLOGICAL LABORATORY, Woods Hole, and at the CALIFORNIA INSTITUTE OF TECHNOLOGY, Pasadena. Preliminary accounts of the results have already been published (DOBZHANSKY 1929a and 1929b).

The author wishes to express his gratitude to Doctor T. H. MORGAN, Doctor A. H. STURTEVANT and Doctor C. B. BRIDGES for their invaluable advice, suggestions and criticism without which the present work could hardly have been done. The author is obliged to Miss E. M. WALLACE for the finishing of the drawings accompanying this paper.

ORIGIN OF THE TRANSLOCATIONS

Males heterozygous for the second-chromosome dominant gene Bristle (B_1 - short bristles), and the third-chromosome dominant gene Dichaete (D - wings spread, anterior dorsocentral bristles missing) were treated by X-rays. The age of the flies at the moment of the treatment was not more than 14 hours from the emergence from the puparia. A glass vial was filled with cotton to within about $1\frac{1}{2}$ cm from the opening; flies were placed on this cotton, and the opening of the vial closed by a cotton stopper. The vial was placed vertically, the opening up, under the target of a broad-focus Coolidge tube, so that the flies were at 16 cm from the target. Between the vial and the tube an aluminum plate of about 1 mm thickness

was inserted. The tube was operated by a current of 50 peak kilovolts and 5 milliamperes during 48 minutes. As seen from these data, the dosage of X-rays used is approximately equal to that applied by MULLER (1928) in his experiments and called "t-4"

The treated males were crossed in individual cultures to untreated females having attached X-chromosomes homozygous for the sexlinked gene yellow (y -yellow body color) and homozygous for the fourth-chromosome recessive gene eyeless² (e_v^2 -eyes small, occasionally absent). Of the 57 cultures in which the fathers were treated, 18 cultures gave no offspring. Of the 10 control cultures (in which untreated males and females of the same genetical constitution as in the treated series were bred) only one culture was sterile. The average number of offspring per culture in the treated series was markedly lower than in the control series. Evidently the dosage of X-rays was sufficient to produce a significant decrease of fertility in the treated males.

In the F_1 generation all the expected classes of offspring appeared in each of the cultures of the treated as well as of the control series. These classes are:

Females:	y ,	yB_i	yD ,	$y B_i D$
Males:	$+$,	B_i ,	D ,	$B_i D$.

Although no particular care was paid to the detection of mutations, some were found in the cultures of the treated series, but none in the control series. Two of the mutations found were extreme allelomorphs of forked, one was a dominant (lethal when homozygous) allelomorph of vestigial (phenotypically like Beaded), and one was an extreme Minute (locus not determined). Besides the mutations, there were found nine individuals which were mosaics having one-half or one-quarter of the body Haplo-IV eyeless. These mosaics were certainly due to the elimination of the treated fourth chromosome in the cleavage of the eggs from which the mosaic individuals developed. The grand total of flies examined in the treated series was 2420, and in the control series - 1067.

Let us consider the genotypical constitution of the $B_i D$ males which appeared in the cultures of the treated series. They received a treated X-chromosome, a treated second chromosome carrying B_i , a treated third chromosome carrying D , and a treated fourth chromosome carrying the normal allelomorph of e_v from their fathers. On the other hand, they received an untreated Y-chromosome, an untreated second and third chromosome carrying normal allelomorphs of B_i and D respectively, and an untreated fourth chromosome carrying e_v (The allelomorph of e_v ,

used throughout the present study is e_v^2 . However, for sake of simplicity, the symbol e_v will be used from now on.) from their mothers. In other words, all the four pairs of chromosomes of these males are "marked" by different characters, namely: sex, B_1 , D and e_v .

From the treated series 153 $B_1 D$ males and from the control series 26 $B_1 D$ males were crossed in single-pair cultures to untreated females homozygous for e_v . The progeny of this cross normally consists of 16 classes of offspring representing all the recombinations of the four "marking" characters mentioned. These classes are:

Females:	$+$, B_1 , D , e_v , $B_1 e_v$, $D e_v$, $B_1 D$, $B_1 D e_v$,
Males	$+$, B_1 , D , e_v , $B_1 e_v$, $D e_v$, $B_1 D$, $B_1 D e_v$.

However, if a parental $B_1 D$ male carries a translocation involving any two chromosomes, the individuals of some of these 16 classes of offspring may be expected either to be missing, or to possess visible external abnormalities. For instance, if the male carries a translocation involving the treated second and third chromosomes, no D , B_1 , $D e_v$ and $B_1 e_v$ individuals of either sex are expected in his progeny. Likewise, if such a male carries a translocation involving the treated second and X-chromosomes, no B_1 males and no non- B_1 females are expected in his progeny (or, B_1 males and non- B_1 females may occur, but be abnormal in appearance).

Of the 144 cultures of the treated series, 23 cultures were sterile; of the 26 control cultures only 2 were sterile. Furthermore, 112 cultures of the treated series and 24 cultures of the control series produced normal progenies, that is, in the overwhelming majority of these cultures all 16 expected classes of offspring were present in approximately equal numbers. Only two of these "normal" cultures produced too small a number of offspring, and failed, therefore, to show one of the expected classes. Table 17 presents the summary of the counts observed in the "normal" cultures of the treated series.

The results obtained in the remaining nine cultures of the treated series are presented in table 1. In five of these cultures (No. 1271, 1318, 1319, 1407, 1425) eight of the expected classes, representing the recombination of D and e_v , are missing. This fact suggests that a translocation involving the third and the fourth chromosomes is present in each of these cultures. In cultures 1223, 1239, 1289 and 1401, eight classes which represent the recombinations of B_1 and D are missing, suggesting the presence of translocations involving the second and third chromosomes.

The results obtained in these nine cultures are, indeed, so strikingly different from the results obtained in all the "normal" cultures, that no

TABLE 1
Cultures showing linkage of genes located in different chromosomes.

CULTURE NUMBER	STRAIN	TRANSLOCATION INVOLVING CHROMOSOMES	WILD TYPE		B_t		D		e_y		$B_t D$		$D e_y$		$B_t e_y$		$B_t D e_y$	
			♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
1223	A	II-III	28	28	24	26	22	24	15	21	
1239	B	II-III	22	16	20	20	28	21	14	19	
1289	C	II-III	4	5	8	4	9	14	9	6	
1401	D	II-III	3	8	7	7	2	4	4	12	
1271	a	III-IV	18	12	11	21	19	18	10	
1318	b	III-IV	14	13	20	15	21	13	12	7	..	
1319	c	III-IV	19	15	9	13	22	19	26	17	..	
1407	d	III-IV	13	7	12	14	10	11	17	12	..	
1425	e	III-IV	24	19	12	15	17	17	13	15	..	

doubt is possible in classifying a given culture as belonging to the "normal" or to the "abnormal" class.

Each of the nine cultures just described served as the progenitor of a separate strain, in which the abnormal genetical situation present in these cultures has been maintained by appropriate breeding. These strains are designated by the characters of the alphabet, as shown in table 1. The present paper deals only with the results of the investigation of the translocations involving the third and the fourth chromosomes. The translocations involving the third and the second chromosomes will be described in a separate paper.

WORKING HYPOTHESES

The genes D and e_y are localized in the third and in the fourth chromosomes respectively. Nevertheless, there is observed a complete linkage between D and e_y in five cultures shown in table 1. The first question concerning the nature of this abnormality is whether the causes which prevented the free recombination of D and e_y in these five cultures are hereditary.

Dichaete non-eyeless males from each of these five cultures were crossed separately to their eyeless sisters. Only D and e_y (no wild-type and no De_y) offspring were reproduced (table 2). In the next generation D

males were crossed to unrelated eyeless females; again only D and e_y individuals were produced. The linkage of D and e_y is, therefore, permanent, and Dichaete individuals transmit the cause producing this linkage to their Dichaete progeny. This fact gives a simple method of maintaining stocks of the translocations: D males from the stock cultures of the translocations are crossed in each generation to their e_y sisters or to unrelated e_y females.

TABLE 2
Progeny of Dichaete-flies (carrying translocations) when crossed to eyeless.

TRANSLOCATION	EYELESS ♀ × DICHAETE ♂					DICHAETE ♀ × EYELESS ♂				
	D	+	De_y	e_y	TOTAL	D	+	De_y	e_y	TOTAL
a	480	424	904	1080	2	2	915	1999
b	349	324	673	818	1	2	689	1510
c	344	303	647	1140	98	61	901	2200
d	313	415	728	540	381	434	645	2000
e	306	275	581	1240	52	58	842	2192

TABLE 3
Bent ♀ × Dichaete ♂ (carrying translocations).

TRANSLLOCATION	D	+	Db_1	b_1	TOTAL
a'	286	—	—	245	531
b	320	—	—	314	634
c	250	—	—	285	535
d	209	—	—	251	460
e	261	2(?)	—	249	512

If D females carrying a translocation are crossed to e_y males, the progeny consists chiefly of D and e_y flies, but some wild-type and De_y individuals also appear (table 2). In males the linkage between D and e_y is complete, but in the females these genes may be separated from each other by crossing over. In other words, in the translocations the genes D and e_y behave as if they were localized in the same chromosome.

The frequency of crossing over between the loci occupied by D and e_y is very different in different translocations. In a- and b-translocations only 0.2 percent of crossing over occurs between D and e_y ; in c- and e-translocations the frequency of crossing over is 7.2 percent and 5.0 percent respectively; in d-translocation a frequency as high as 40.7 percent was observed.

Two hypotheses explaining the behavior of D and e_v in the translocations may be suggested. The first hypothesis is that one of the fourth chromosomes of the treated male carrying the normal allelomorph of e_v has become permanently attached to the D -carrying third chromosome. Hence, from such a male all the offspring receiving the D -carrying third chromosome receive at the same time the fourth chromosome containing the normal allelomorph of e_v , and consequently are Dichaete non-eyeless in appearance. However, in females carrying translocations, D and e_v may be separated by crossing over; hence, wild-type and $D e_v$ individuals appear along with D and e_v in the progeny. The frequency of the crossing over between D and e_v depends upon the distance between the locus of D and the point of attachment of the fourth chromosome to the third chromosome. Since the frequency of this crossing over is found to be very diverse in the five translocations studied, the fourth chromosome may be assumed to be attached to different parts of the third chromosome.

The second hypothesis assumes that a broken off section of the D -carrying third chromosome from the treated male is attached to the fourth chromosome carrying the normal allelomorph of e_v . In other words, the third chromosome was broken into two fragments, and one of these fragments became attached to the fourth chromosome. On this hypothesis males carrying a translocation produce two kinds of gametes in equal numbers (see figure 1). One kind of gametes contains both fragments of the third chromosome, one fragment being attached to the fourth chromosome. These gametes carry, therefore, the gene D and the normal allelomorph of e_v . The other kind of gametes contains the normal (that is, the unbroken) third chromosome and the free fourth chromosome. These gametes carry e_v but do not carry D . If such a male is crossed to a female homozygous for e_v but free from the translocation, only Dichaete non-eyeless and eyeless non-Dichaete individuals are produced (see figure 1). On the other hand, in females carrying a translocation both fragments of the third chromosome involved in the translocation may conjugate with the unbroken third chromosome. If crossing over occurs after such a synapsis, D and the normal allelomorph of e_v may be separated from each other. Consequently, $D e_v$ and wild-type individuals may appear in the progeny of a female carrying a translocation.

The two hypotheses just outlined are rather similar in nature. Both of them involve the assumption that in the translocations a union between the third and the fourth chromosomes occurred. According to both of them the frequency of crossing over between D and e_v indicates the distance between the locus of D and the locus of the attachment of the fourth chromo-

some. The only essential difference between these two hypotheses is that the first of them postulates the attachment of the fourth chromosome to the unbroken third chromosome, while the second hypothesis postulates a breakage of the third chromosome and an attachment of the fourth chromosome to one of the resulting fragments of the third. This difference is, however, important from the standpoint of terminology. If the fourth chromosome is attached to the unbroken third, the phenomenon should be

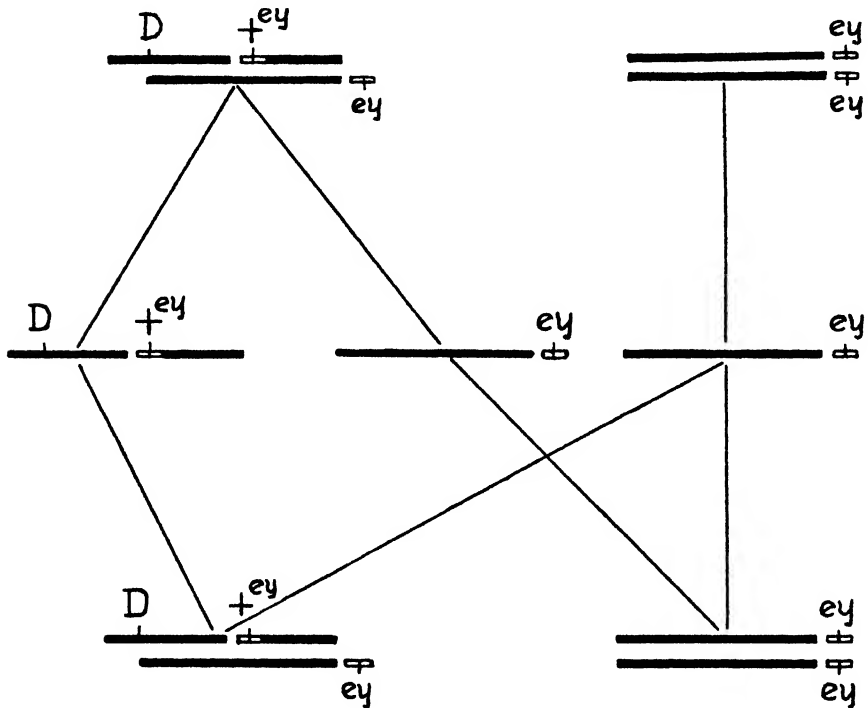


FIGURE 1.—Scheme of a mating of a fly heterozygous for a translocation to a fly free from translocations. Third chromosome—black; fourth chromosome—white. *D*—Dichaete; *ey*—eyeless. Only gametes giving rise to viable zygotes are represented in this figure.

termed not a translocation but a compounding of chromosomes (see BRIDGES 1923). However, if a fragment of the third chromosome is attached to the fourth, we are dealing with a translocation of a section of the third chromosome on to the fourth.

The cytological investigation is, obviously, the most direct way to the solution of these alternatives. However, before presenting the results of the cytological investigation of the translocations, their genetical behavior may be examined in fuller detail.

BEHAVIOR OF THE GENES OF THE FOURTH LINKAGE GROUP
IN THE TRANSLOCATIONS

The fourth chromosome of *Drosophila melanogaster* is much smaller in size than the other chromosomes of this species. In accordance with this smallness, the number of genes localized in the fourth chromosome is also much less than the number of the genes in other chromosomes. Furthermore, no (or very little) crossing over takes place between the known genes of the fourth chromosome (unpublished data of BRIDGES). It seems *a priori* very probable that in translocations the behavior of all the fourth chromosome genes is identical with the behavior of e_y , which has been discussed above. Both hypotheses advanced in the preceding section assume that in all the translocations the whole fourth chromosome rather than a fragment of it is attached to the third chromosome. The extreme smallness and the absence of crossing over in the fourth chromosome make the contrary supposition improbable.

To test the validity of this point, D males carrying translocations were crossed to females homozygous for bent. Bent (b_t) is a fourth-chromosome recessive gene producing a shortening of the legs and a spreading of the wings of flies. From the F_1 of this cross D males were selected and back-crossed to homozygous b_t females. In the next generation only D and b_t (no wild-type and no $D b_t$) individuals appeared (table 3). The two wild-type flies shown in table 3 were probably genotypically b_t ; their appearance is due to the fact that b_t some times overlaps wild type phenotypically. When D females from the F_1 were crossed to b_t males, some wild-type and $D b_t$ individuals appeared along with D and b_t individuals. The behavior of b_t in translocations is, therefore, identical with behavior of e_y .

The presence of a linkage between D and the fourth-chromosome dominant gene Minute-IV in translocations was proved by a similar method. Dichaete males carrying translocations were crossed to females heterozygous for Minute-IV (Minute-IV is lethal when homozygous). In the F_1 generation Dichaete Minute-IV males were selected and crossed to wild-type females. Only D and Minute-IV (no wild type and no Dichaete Minute-IV) were produced in the progeny.

Although the two remaining genes located in the fourth chromosome (namely shaven and rotated abdomen) were not tested in the translocations, there can be no doubt that their behavior is identical with the behavior of e_y , b_t and Minute-IV.

CROSSING OVER IN THE THIRD CHROMOSOME
INVOLVED IN THE TRANSLOCATIONS

A series of experiments was necessary to determine the exact loci in the third chromosome at which the fourth chromosome is attached in the different translocations. The exact determination of these loci is especially important, since, according to the second hypothesis outlined above, they may coincide (and as it will be proved later do coincide) with the breaking-points of the third chromosome in the translocations.

Dichaete males carrying translocations were crossed to females homozygous for e_y and homozygous for the third-chromosome recessive genes roughoid (r_u -rough eye-surface), hairy (h -hairs along the wing-veins), thread (t_h -unbranched aristae), scarlet (s_t -bright red eye-color), curled (c_u -wings curved upwards), stripe (s_r -dark longitudinal stripe on the thorax), sooty (e^s -dark body-color) and claret (c_a -purplish pink eye-color). Dichaete females were selected in the F_1 generation of this cross and backcrossed to $e_y r_u h t_h s_t c_u s_r e^s c_a$ males. The results obtained in the next generation are presented in tables 11-15 (appendix). (Flies homozygous for the allelomorph of e_y used, namely e_y^2 , rarely have both eyes completely absent. In flies having no eyes the classification in respect to r_u , s_t and c_a is, of course, impossible. Such flies were disregarded and are not included in the counts presented in tables 11-15. The frequency of homozygous e_y^2 flies having no eyes is, however, so low that their omission can not influence appreciably the crossing over values obtained.)

The frequency of crossing over in an interval between two given genes depends to a certain extent upon the modifying genes present in the stock used. Therefore, it is not desirable to compare the frequency of crossing over in the third chromosome involved in the translocations directly with the frequency of crossing over in the normal third chromosome indicated by the standard map of this chromosome (for the standard map see figure 2, also MORGAN, BRIDGES, STURTEVANT 1925, p. 92). With the object of having a better standard of comparison a special control experiment was made. Dichaete males from the same stock of D flies from which the D males treated by X-rays were obtained (see above) were crossed to homozygous $e_y r_u h t_h s_t c_u s_r e^s c_a$ females from the same stock which was used for the study of crossing over in the translocations. In the F_1 generation D females were selected and backcrossed to $e_y r_u h t_h s_t c_u s_r e^s c_a$ males. The results obtained in the next generation are recorded in table 16 (appendix).

The crossing over values were calculated from the data shown in tables 11-16. The calculated crossing over values are presented in table 4. As

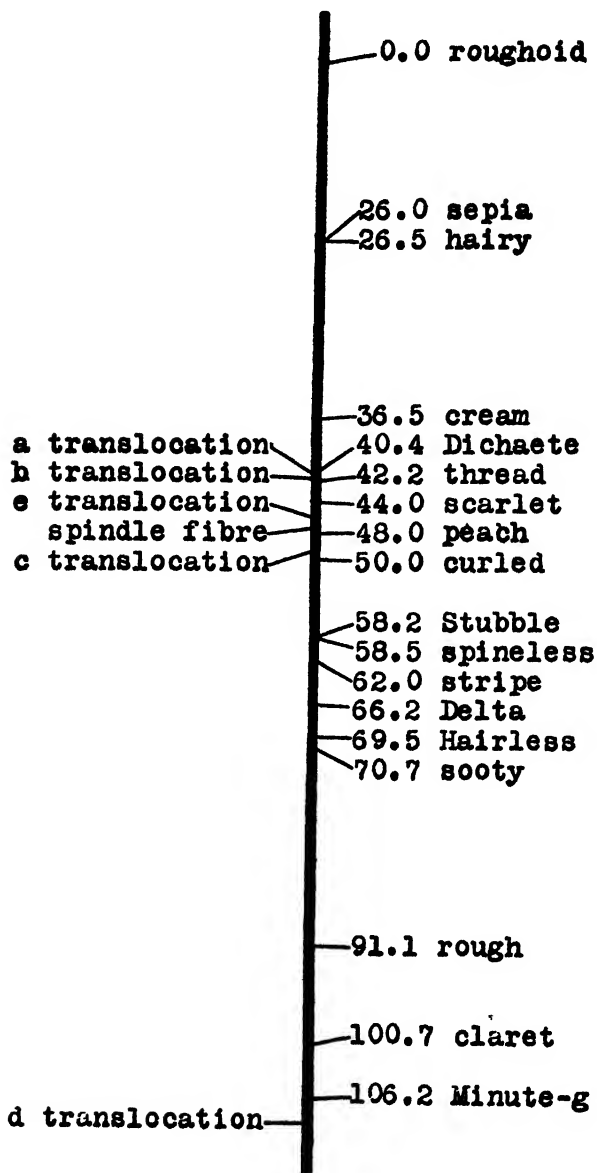


FIGURE 2.—Genetical map of the third chromosome of *Drosophila melanogaster*.

seen from table 4, the point of attachment of the fourth chromosome to the third (designated in table 4 by +) is not alike in the different translocations.

In a-translocation this point is located between the genes D and t_h . In b-translocation the point of attachment is nearly the same as in a-translocation but in b-translocation it seems to coincide with the locus of the gene t_h . In c-translocation and in e-translocation the fourth chromosome is attached near the middle of the length of the third chromosome, namely in the s_t-c_u interval. However, in e-translocation the point of attachment is located nearer to s_t than to c_u , whereas in c-translocation it is nearer to c_u than to s_t . Finally, in d-translocation the fourth chromosome is attached near the extreme right-hand end of the third chromosome, 5.1 units to the right of the gene c_a .

TABLE 4
Crossing over values for the intervals studied in the third chromosome.

Translocation a.									
Interval	r_u-h	$h-D$	$D-+$	$+t_h$	t_h-s_t	s_t-c_u	c_u-s_r	s_r-e^a	e^a-c_a
Value	17.2	2.9	.05	.1	.2	6.6	16.6	11.7	31.0
Translocation b.									
Interval	r_u-h	$h-D$	$D-+$	$+t_h$	t_h-s_t	s_t-c_u	c_u-s_r	s_r-e^a	e^a-c_a
Value	21.2	5.9	.2	.3	4.6	17.1	10.0	31.0	
Translocation c.									
Interval	r_u-h	$h-D$	$D-t_h$	t_h-s_t	s_t-+	$+c_u$	c_u-s_r	s_r-e^a	e^a-c_a
Value	26.9	15.4	1.5	.9	3.8	.7	3.7	7.5	28.9
Translocation d.									
Interval	r_u-h	$h-D$	$D-t_h$	t_h-s_t	s_t-c_u	c_u-s_r	s_r-e^a	e^a-c_a	c_a-+
Value	29.1	14.8	1.1	.9	5.9	12.6	8.9	19.2	5.1
Translocation e.									
Interval	r_u-h	$h-D$	$D-t_h$	t_h-s_t	s_t-+	$+c_u$	c_u-s_r	s_r-e^a	e^a-c_a
Value	19.5	11.6	1.1	.5	1.5	3.6	15.6	11.6	31.0
Control									
Interval	r_u-h	$h-D$	$D-t_h$	t_h-s_t	s_t-c_u	c_u-s_r	s_r-e^a	e^a-c_a	
Value	23.7	13.7	1.1	.8	8.0	15.4	10.3	31.0	

Comparison of the crossing over values for the different intervals of the third chromosome observed in the translocation and in the control experiment reveals the presence of consistent differences between them (see table 5). In a, b- and in e-translocations the frequency of crossing over is markedly decreased to the left of the s_t-c_u interval but slightly increased to the right of this interval. On the other hand, in c- and d-translocations crossing over is decreased to the right of the s_t-c_u interval but increased to the left of it.

These regularities may be described also as follows. A point located approximately in the middle of the s_t-c_u interval divides the third chromosome into two limbs. If the fourth chromosome is attached to the third

chromosome to the left of this point (as is the case in a-, b- and e-translocations), the frequency of crossing over is decreased in the left limb but increased in the right limb of the third chromosome. If, however, the point of the attachment of the fourth chromosome lies to the right of the point mentioned above (as is the case in c- and d-translocations), the frequency of crossing over is decreased in the right limb of the third chromosome but increased in the left limb (see figure 2). (Following the practice established in the *Drosophila* literature, the end of the third chromosome at which the gene c_a is located is designated in this discussion as the right-hand end, and the end at which the gene r_u is located as the left-hand end of this chromosome.)

TABLE 5

Differences between the crossing-over values in the translocations and the corresponding values observed in the control experiment.

INTERVAL	TRANSLLOCATION					
		a	b	c	d	e
Left of the spindle fibre	r_u-h	-6.5	-2.5	+3.2	+5.4	-4.2
	$h-D$	-10.8	-7.8	+1.7	+1.1	-2.1
	$D-t_h$	-.95	-.9	+.4	0.0	0.0
	t_h-s_t	-.6	-.5	+.1	+.1	-.3
Spindle fiber	s_t-c_u	-1.4	-3.4	-3.5	-2.1	-2.9
Right of the spindle fibre	c_u-s_r	+1.2	+1.7	-11.7	-2.8	+.2
	s_r-e^a	+1.4	-.3	-2.8	-1.4	+1.3
	e^a-c_a	0.0	0.0	-2.1	-11.8	0.0

The strongest reduction of the frequency of crossing over is observed in the intervals adjacent to the locus of the attachment of the fourth chromosome. Nevertheless, some reduction of crossing over takes place in all the intervals of the limb of the third chromosome in which the point of attachment of the fourth chromosome lies. For instance, in d-translocation the greatest decrease of the frequency of crossing over is observed in the e^a-c_a interval, while in the c_u-s_r interval the reduction is comparatively slight. In d-translocation the point of attachment of the fourth chromosome lies near the e^a-c_a interval. On the contrary, in c-translocation the frequency of crossing over is strongly decreased in the c_u-s_r interval, while in the e^a-c_a interval it is but slightly affected. In c-translocation the fourth chromosome is attached to the left of c_u . It is significant, however, that if the attachment of the fourth chromosome is near to the point dividing the third chromosome in two limbs (for example, the point lying half way between s_t and

c_u) the decrease of the crossing over frequency does not affect the adjacent intervals of both limbs (see c- and e-translocations). This fact suggests that the point dividing the two limbs of the chromosome plays some especially important role in the distribution of the crossing over along the third chromosome.

Most of the differences between the crossing over values for the respective intervals observed in the translocations and in the control experiment are statistically significant. Furthermore, the consistency of the results obtained suggests that these differences are significant even in the cases where the value of the difference in question is below the limit of statistical certainty.

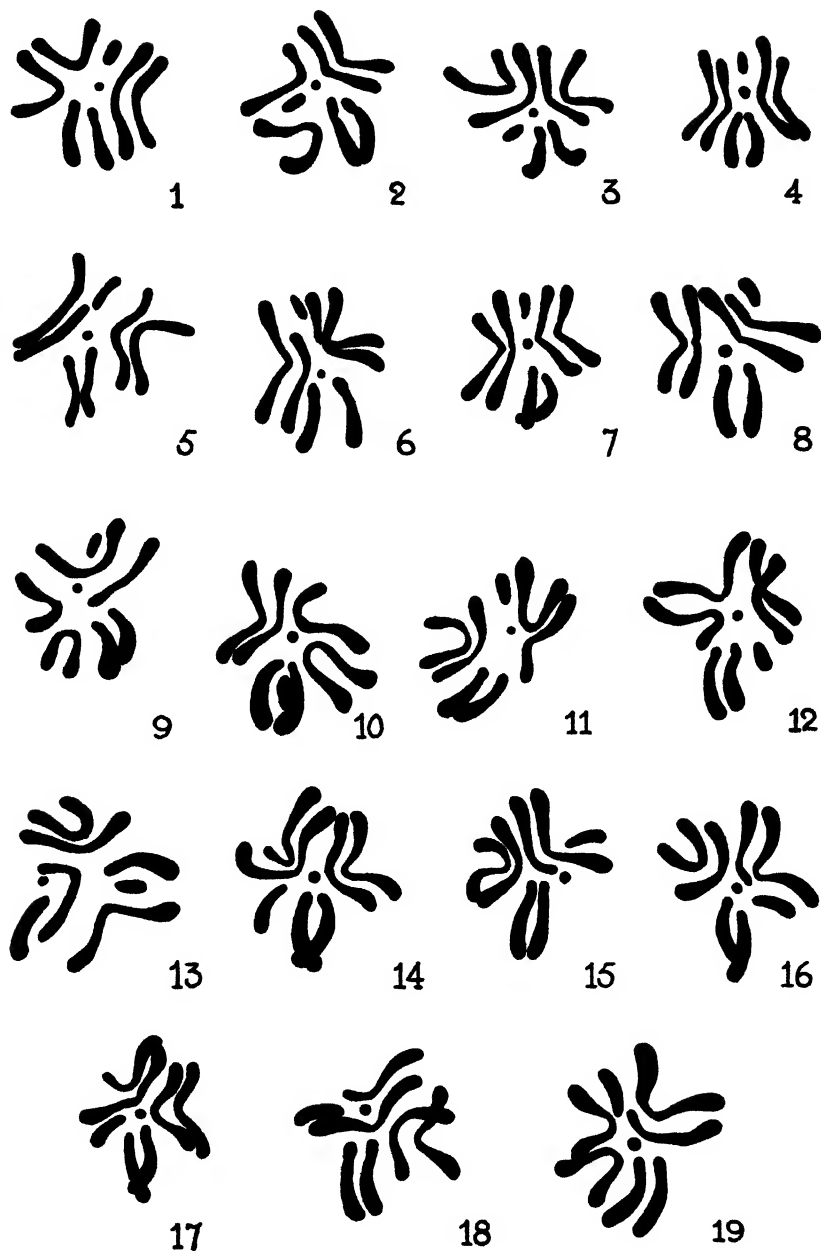
It might be supposed that differential viability of the various classes of crossover individuals is responsible for the alteration of the crossing over frequencies in the translocations. Since the point of attachment of the fourth chromosome to the third is not the same in different translocations the distribution of e_v among the crossovers is not alike in different cases. The gene e_v in homozygous condition causes some decrease of the viability of the flies. However, the difference between the crossing over values found in the different translocations can not be accounted for by the influence of e_v on the viability of the flies. Indeed in both c- and e-translocations the locus of the fourth chromosome attachment lies in the $s_t - c_u$ interval. The distribution of e_v among the crossing-over individuals is, therefore, alike in c- and e-translocations. Nevertheless, in c-translocation there is observed a decrease of the frequency of crossing over in the right limb and an increase in the left limb of the chromosome, while in e-translocation a decrease takes place in the left limb and an increase in the right limb.

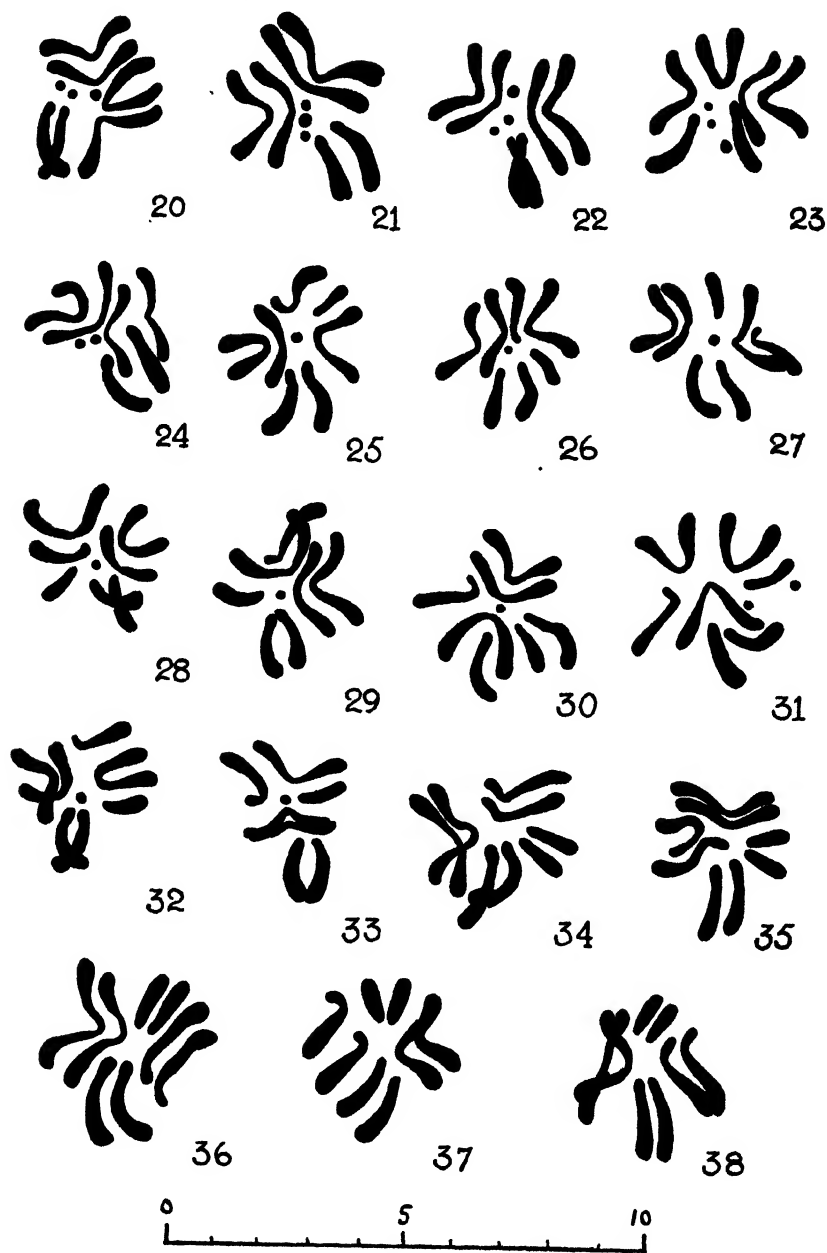
The third chromosome of *Drosophila melanogaster*, as seen in the cytological preparations, is V-shaped, and the point of attachment of the spindle fibre to the body of the chromosome lies at the middle of its length, dividing the chromosome into two limbs of equal size. It is very probable that the decrease of the frequency of crossing over in one part of the chromosome and the increase of its frequency in the other part of the same chromosome is due to the location of the point of the attachment of the fourth in relation to the spindle fibre. If the fourth chromosome is attached to the left of the locus of the attachment of the spindle fibre (in a-, b- and e-translocations) crossing over is reduced in the left limb of the chromosome and increased in the right limb. If, however, the fourth chromosome is attached to the right of the spindle fibre (as in c- and d-translocations), the crossing over frequency is decreased in the right limb but increased in

EXPLANATION OF PLATES 1 AND 2

The drawings represent chromosomes (oögonial plates) of the females carrying translocations in heterozygous (figures 1-33) or in homozygous (figures 34-38) condition. All the drawings are done at the level of the work-table with the aid of camera lucida, under the Zeiss immers. obj. 1.5 and comp. oc. 30. The scale given in plate 2 below, common to all the drawings, represents 10 micra. As far as possible the figures were placed so that the X-chromosomes occupy the lower part of each figure.

Plate 1, figures 1-6 represent a-translocation; figures 7-13 b-translocation; figures 14-19 c-translocation (heterozygous); plate 2, figures 20-24 d-translocation; figures 25-33 e-translocation; figures 34-38 homozygous c-translocation.





the left limb of the chromosome. It is a plausible assumption that the point lying half way between s_t and c_u which divides the third chromosome in two "genetical limbs" (see above) is in reality the point of spindle-fibre attachment. The location of this point approximately in the middle of the length of the map of the third chromosome (see figure 2) is in good agreement with such an assumption, since the attachment of the spindle fibre is located cytologically also at the middle of the length of the chromosome.

It must be emphasized that the location of the point of spindle-fibre attachment between the loci of s_t and c_u was already probable on the basis of the data secured by quite independent methods. BRIDGES and MORGAN (1923) on the basis of the study of the distribution of double crossing over in the third chromosome came to the conclusion that the point of the spindle-fibre attachment lies between the loci of s_t and peach (the locus of peach is between s_t and c_u). STURTEVANT came to the same conclusion on the basis of the study of inverted sections (STURTEVANT and PLUNKETT 1926, also unpublished data). Recently REDFIELD (unpublished) obtained further evidence pointing in the same direction, from investigations of the crossing over frequency in the third chromosome of triploid females.

It has been shown above that in c-translocation the frequency of crossing over is decreased in the right limb and increased in the left limb of the third chromosome, while in e-translocation the conditions are reversed. It follows that the point of spindle-fibre attachment lies between the loci of attachment of the fourth chromosome in c- and in e-translocation. It is known, furthermore, that in both c- and e-translocations the fourth chromosome is attached to the third between the genes s_t and c_u . Therefore it becomes especially important to determine the location of the fourth-chromosome attachment in c- and e-translocations in respect to the gene peach (p -eye color) which lies within the s_t - c_u interval.

TABLE 6
 e_y D
 Crossing over in ——— $\varphi \times e_y D s_t p c_u \sigma^7$.
 .. $s_t p c_u$

		Translocation c.			Total flies examined
Interval	$D-s_t$	s_t-p	$p+$	$+c_u$	
Value	2.2	3.4	.07	.2	1481
		Translocation e.			Total flies examined
Interval	$D-s_t$	s_t+	$+p$	$p-c_u$	
Value	.8	1.2	1.1	2.4	1288

Dichaete males carrying c- and e-translocations respectively were crossed to females homozygous for e_y , s_t , p and c_u . In the F_1 generation D females were selected and backcrossed to $e_y s_t p c_u$ males. The results

obtained in the next generation are summarized in table 6. As seen from table 6, in e-translocation the locus of attachment of the fourth chromosome lies in the s_1 - p interval while in c-translocation it lies in the p - c_u interval. The frequency of crossing over in the D - s_1 and the s_1 - p intervals is considerably lower in e-translocation than in c-translocation. On the contrary, the frequency of crossing over in the p - c_u interval is lower in c-translocation than in e-translocation.

It may be concluded on the basis of this evidence that the point of the spindle-fibre attachment is located near the gene peach, probably slightly to the left of it. In other words, the spindle fibre is attached to the third chromosome between the loci 46 and 48 of the standard map (see figure 2).

CYTOLOGICAL CHANGES PRODUCED BY THE TRANSLOCATIONS

It has been pointed out above that the genetical behavior of the translocations may be explained on either of two assumptions. First, the fourth chromosome may have become permanently attached to the third chromosome. Second, a section of the third chromosome may have become broken off and attached to the fourth chromosome. The observed alterations of the frequency of crossing over in the the third chromosome might be produced, judging *a priori*, in either case. In other words, the genetical data so far presented give no evidence as to which of these two assumptions is correct. This, however, may be easily decided by the cytological investigation of the translocations. If the fourth chromosome in the translocations is attached to the unbroken third, only one instead of two fourth chromosomes must be present in the chromosomal plates. Except this, the chromosomes must look normal, since the point of the attachment of the fourth chromosome will be, probably, indistinguishable because of the extreme smallness of this chromosome. However, if a section of the third chromosome is broken off and attached to the fourth chromosome, one of the cytologically visible third chromosomes must be shortened, and one of the fourth chromosomes must be increased in size because of the addition of the material from the third chromosome.

Ovaries of freshly hatched females or of mature pupae were dissected and fixed successively in strong Flemming's fluid (1 hour) and in 1 percent solution of chromic acid (20 hours). Not less than 7 sufficiently clear chromosome plates were obtained for each of the five translocations. Most of them are presented in plates 1 and 2 (see also figures in DOBZHANSKY 1929a).

It is most convenient to begin the presentation of the results of the cytological investigation of the translocations with a description of the features in common to a-, b-, c- and e-translocations (plate 1, figures 1-19,

plate 2, figures 25–33). The overwhelming majority of the chromosomal plates studied in these translocations (52 plates out of 54 studied) contain only one free fourth chromosome of normal size. This fact is important, since it may be considered as a cytological proof of our basic assumption, namely that the observed linkage between the genes belonging to the third and to the fourth linkage groups is due to translocations involving these chromosomes. Individuals having only one fourth chromosome are known in *Drosophila melanogaster*, but they always have a complex of external peculiarities called “Haplo-IV” or “Diminished” (BRIDGES 1921). However, flies carrying translocations never possess the characteristics of Haplo-IV. This fact may be explained only on the assumption that the flies carrying translocations have in reality two fourth chromosomes, one free, and the other attached to the third chromosome or to a fragment of the latter. The few cases in which two free fourth chromosomes are found in the translocations are probably due to non-disjunction of the fourth chromosomes. In these cases the individuals have in reality three fourth chromosomes, two of them being free and one attached to the third chromosome. The plausibility of this explanation will be examined below.

The pair of X-chromosomes and the smaller pair of the V-shaped autosomes appear to be perfectly normal in all plates. However, the larger pair of the V-shaped autosomes consists of two partners distinctly unequal in length, although showing somatic pairing with each other in the majority of the plates studied. The longer partner is symmetrical, that is, it has both limbs equally long. The equality of the length of the limbs of the longer partner is especially clearly seen in the cases in which the median constriction of the chromosome is clearly pronounced (see plate 1, figures 6, 7, 8, 12, 16, 17, 18, plate 2, figures 27, 31). On the other hand, the shorter partner is asymmetrical, that is, it has one of the limbs markedly shorter than the other. The inequality of the two limbs of the asymmetrical partner is manifest in all plates studied, but it is especially striking in the case where the median constriction is pronounced (see plate 1, figures 3, 7, 10, 14, plate 2, figures 27, 29, 33).

Since the V-shaped autosomes in the chromosomal plates of the normal flies have both limbs equal in length, it is possible to conclude that the asymmetrical autosomes just described are the third chromosomes involved in the translocations.

The asymmetrical chromosome is the member of the larger pair of the V-shaped autosomes. The difference in length between the larger and the smaller pair of the V-shaped autosomes is clear enough in the majority of

the chromosomal plates studied. Among all the plates presented here perhaps only those in figure 5 (plate 1) and in figure 23 (plate 2) may be uncertain as to which of the two pairs of the V-shaped autosomes is the larger and which the smaller. Therefore, it is possible to conclude that the larger pair of the V-shaped autosomes of *Drosophila melanogaster* carries the third-chromosomal linkage group of genes, whereas the smaller pair carries the second-chromosome linkage group. In what follows we shall call the larger pair of the V-shaped autosomes the third chromosome, and the smaller pair the second chromosome.

This identification of the third and the second chromosomes is in disagreement with the identification given by BRIDGES (MORGAN, STURTEVANT, BRIDGES 1928), MULLER and PAINTER (1929) and PAINTER and MULLER (1929). Indeed, the authors mentioned identified the smaller pair of the V-shaped autosomes in *Drosophila melanogaster* as being the carrier of the third-chromosome linkage group, and the larger pair as the carrier of the second-chromosome linkage group of genes. The conclusions of BRIDGES were based on the cytological study of "Pale" translocation in which case only a very small fragment of the second chromosome has been broken off and attached to the third chromosome. The cytological differences observed were so slight, that no decisive conclusion could be drawn. In fact, BRIDGES himself previously identified the larger pair as the third chromosome and the smaller pair as the second chromosome (MORGAN, BRIDGES, STURTEVANT 1925, p. 177) on the basis of the study of the same "Pale" translocation. Doctor C. B. BRIDGES has seen my preparations and agrees with my interpretation. MULLER's and PAINTER's material is quite dependable, since the translocations described by these authors involve large sections of the second and third chromosomes. But, as far as I can see, at least some of PAINTER's own figures representing the cytological conditions in the translocations involving the second or the third chromosomes show the third chromosome longer than the second (see MULLER and PAINTER 1929, figures 1, 3, 5, 6, PAINTER and MULLER 1929, figure 29). As far as the length of the chromosomes is concerned there remains no doubt that the third chromosome is longer than the second.

Both ends of the normal V-shaped autosomes are thicker than the middle part of the same chromosomes. It is worth notice that the shorter limb of the asymmetrical third chromosome found in the translocations is always less thickened at the end than the longer limb of the same chromosome. The longer limb of the asymmetrical third chromosome is equal

in length and shape to either limb of the normal third chromosome present in the same plate.

Obviously a loss of some material normally located in the third chromosome is responsible for the shortening of one of the limbs of the asymmetrical third chromosome. This material is undoubtedly to be looked for in the small, rod-shaped, unpaired chromosome clearly visible in all the chromosomal plates in the translocations but not present at all in the normal chromosomal plate of *Drosophila melanogaster*. The length of the rod-shaped chromosome plus the length of the asymmetrical third chromosome is about equal to the length of the normal third chromosome present in the same plate. One of the ends of the new rod-shaped chromosome (that directed toward the periphery of the chromosomal plate) is in most cases thicker than the other end of the same chromosome (directed toward the center of the plate). The thickness of the thicker end of the rod-shaped chromosome is approximately equal to (or a little smaller than) the thickness of the ends of the normal third chromosome.

The interpretation of the chromosomal relations found in a-, b-, c- and e-translocations is obvious. In each of these translocations a third chromosome is broken into two fragments of unequal length. The shorter of these fragments is attached to a fourth chromosome. The resulting compound-chromosome (a fragment of the third chromosome plus the fourth chromosome) is seen cytologically as the unpaired rod-shaped chromosome just described. The longer of the fragments is represented by the asymmetrical third chromosome, having one limb markedly shorter than the other limb. The end of the shorter limb of the asymmetrical third chromosome corresponds to the point at which the third chromosome has been broken. Finally, since one of the fourth chromosomes is included in the unpaired rod-shaped chromosome, only, one free fourth chromosome is seen in most of the plates. In other words, the second of the two hypotheses suggested for the explanation of the genetical behavior of the translocations is confirmed by the cytological data.

According to the genetical data presented in the preceding section, the points at which the breakage of the third chromosome occurred in a-, b-, c- and e-translocations are different (see figure 2). If the genetical map represents the real arrangement of the genes in the third chromosome, the broken off sections are of different lengths in different translocations. Consequently, the size of the asymmetrical third chromosome as well as the size of the unpaired rod-shaped chromosome must be differentiated in the different translocations studied.

In a- and b-translocations the third chromosome is broken between the loci of the genes D and t_h ; in b-translocation the breakage occurred very slightly nearer to the middle of the chromosome than in a-translocation. The cytological conditions found in a- and b-translocations are nearly alike (a-translocation plate 1, figures 1–6, b-translocation plate 1, figures 7–13). The length of the unpaired rod-shaped chromosome equals about one-third the length of the limb of the normal third chromosome. The asymmetrical third chromosome has one limb shorter than the other by approximately one-third of its length. In b-translocation the rod-shaped chromosome seems to be very slightly longer than in a-translocation, and correspondingly the asymmetrical third chromosome is slightly shorter in b-translocation than in a-translocation. According to the genetical data the rod-shaped chromosome in these cases must carry the genes from D to the left end of the third chromosome (including the gene r_u), and the asymmetrical third chromosome must carry the genes from t_h to right end of the third chromosome (including c_u).

In e-translocation the third chromosome is broken between the locus of s_i and the hypothetical locus of the spindle-fibre attachment. Consequently, the rod-shaped chromosome in this case contains the genes from s_i to r_u , and therefore must be longer than the rod-shaped chromosome in a- and b-translocations (compare figure 2). The asymmetrical third chromosome in e-translocation carries the genes from p to c_u , and, therefore, its shorter limb must be considerably shorter than in a- and b-translocations. This expectation is confirmed by the cytological investigation of e-translocation (plate 2, figures 25–33). The length of the rod-shaped chromosome equals five-sixths or four-fifths the length of the limb of the normal third chromosome. The asymmetrical third chromosome has one limb very short. It is clearly visible, in cases in which the median constriction is pronounced, that the third chromosome is broken very near its middle, that is, near the point of attachment of the spindle fibre (see figures 27, 29, 33).

In c-translocation the third chromosome is broken between the genes p and c_u , that is to the other side of the spindle-fibre attachment as compared with e-translocation. The rod-shaped chromosome in this case contains the genes from c_u to c_a , and the asymmetrical third chromosome carries the genes from p to r_u . Cytologically (plate 1, figures 14–19) the conditions found in c-translocation are similar to those in e-translocation, but here the rod-shaped chromosome is slightly shorter and the asymmetrical third chromosome slightly longer than in e-translocation. This fact indicates

that the locus of attachment of the spindle fibre is cytologically nearer to the point at which the third chromosome is broken in e-translocation than to the point of breakage in c-translocation.

The chromosomes in d-translocation are at first glance like those of a normal fly. Both pairs of V-shaped autosomes as well as the X-chromosomes appear to be normal. However, one of the fourth chromosomes is clearly increased in size, being roughly from $1\frac{1}{2}$ to twice as large as the other fourth chromosomes present in the same plates. Only in a single plate (figure 24) is the increase in size of one of the fourth chromosomes not distinct. There are present three free fourth chromosomes in most plates in d-translocation, indicating the high frequency of non-disjunction of the fourth chromosomes in this translocation.

The increase in size of one of the fourth chromosomes indicates that the nature of d-translocation is the same as the nature of the other translocations studied, namely that here also a section of the third chromosome is broken off and attached to the fourth chromosome. However, in D-translocation the section of the third chromosome removed from its normal location is so small that its loss is not noticeable in the cytologically visible third chromosome because the third chromosome itself is relatively very large. Nevertheless, the addition of this section to the fourth chromosome is visible cytologically because of the extreme smallness of the latter chromosome.

This explanation agrees very nicely with the genetical data on d-translocation. According to the genetical data the point of breakage of the third chromosome in d-translocation is located 5.1 units to the right of c_a , that is, near the extreme right-hand end of the third chromosome. The map of the third chromosome shows the gene Minute-g located 5.5 units to the right of c_a (figure 2). However, in d-translocation the break in the third chromosome is probably to the right of Minute-g, for the crossing over value for the c^+-c_a interval is 19.2 percent in d-translocation instead of 31.0 units observed in the control. Since in d-translocation the frequency of crossing over is considerably decreased in the right limb of the chromosome, the claret - Minute-g interval is probably considerably shorter than 5.5 units. Correspondingly, the point of the breakage of the third chromosome is located to the right of Minute-g, in the unknown part of the extreme right-hand end of the third chromosome. The size of the section of the third chromosome which has been broken off and attached to the fourth chromosome must be very small in d-translocation.

It is a remarkable fact that in all five cases of translocations studied there is observed a breakage of the third chromosome and an attachment

of the broken off section to the fourth chromosome. In not a single case is the fourth chromosome attached to the unbroken third chromosome. Furthermore, the fragment of the third chromosome attached to the fourth never includes the region of the third chromosome in which the spindle-fibre attachment lies. On the contrary, the fragment of the third chromosome remaining free always includes the region of the spindle-fibre attachment.

These relations suggest the possible manner of origin of the translocation described. The chromosomes treated by X-rays are likely to stick together because of the increase of ionization produced by the rays. An attachment of the fourth chromosome to the third may occur. However, the resulting compound has two spindle fibres (one contributed by the third chromosome and the other by the fourth chromosome). The two spindle fibres may pull the compound chromosome to opposite poles of the spindle during mitosis, producing the breakage of the chromosome, or else the rate of contraction of the two spindle fibres may be different, in which event the breakage of the compound-chromosome also may take place. Both resulting fragments have one and only one spindle fibre, and therefore behave during mitosis like normal chromosomes. It seems very probable that a fragment of a chromosome can be normally transmitted from one cell-generation to the other only when it has its own spindle fibre, or acquires a spindle fibre by an attachment to another chromosome or to a fragment of a chromosome possessing a spindle fibre.

The points of breakage of the third chromosome observed in the translocations do not show a tendency to coincide with the constrictions in the chromosome described by BRIDGES (1927). According to BRIDGES, the third chromosome of *Drosophila melanogaster* has a strong median constriction at the point of the spindle-fibre attachment, and two other less pronounced constrictions in each of the two limbs. One of the latter constrictions, the submedian, lies at about one-fifth of the limb-length from the median constriction; another, still less pronounced, lies at about one-quarter of the limb-length from the end of the chromosome. None of the observed breakages of the third chromosome coincide with the median constriction. The other two constrictions are so inconspicuous that it is difficult to decide whether in a given case the point of breakage coincides with one of them. However, the breakage point in both c- and e-translocations can not coincide with the submedian constriction. Indeed, in c-translocation the point of breakage of the chromosome is located further from the median constriction than in e-translocation, whereas

the submedian constrictions in both limbs of the chromosome are equidistant from the median constriction. Likewise, the point of breakage either in a- or in b-translocation may be supposed to coincide with the constriction located at one-fourth of the limb-length from the end of the chromosome. However, at least in one of these cases the point of breakage certainly does not coincide with the constriction, since, as the genetical data show, the breakages in a- and b-translocations are not located at the same point. Finally, the breakage observed in d-translocation is located in a region of the third chromosome in which no constrictions are known.

GENETICAL CONSEQUENCES OF THE BREAKAGE OF THE THIRD CHROMOSOME IN THE TRANSLOCATIONS

In each of the translocations the locus in the third chromosome at which the fourth chromosome is attached was determined by studying the linkage relations of the genes of the third and fourth linkage groups. It has been pointed out above that this method gave no evidence on the question whether the fourth chromosome is attached to the unbroken third, or a section of the third chromosome has been broken off and attached to the fourth chromosome. This question was solved by the cytological investigation of the translocations. Indeed, in each of the five translocations studied the third chromosome was observed to be broken into two fragments and the shorter of these fragments is attached to the fourth chromosome. It is obviously desirable to check the results of the cytological investigation by an *experimentum crucis*, that is, to demonstrate by purely genetical methods the presence of a break of the third chromosome in the translocations.

As shown by the cytological study, one normal (unbroken) third chromosome, one third chromosome broken into two fragments and one free fourth chromosome are present in each of the cells of the flies carrying translocations. Another fourth chromosome is also present in each of the cells, but it is attached to the shorter fragment of the third chromosome, and therefore is not distinguishable cytologically (see figures 1 and 3). Let us consider the distribution of these chromosomes at the reduction during the gametogenesis.

The unbroken third chromosome and the free fourth chromosome might go to one pole of the spindle at the reduction division, and the two fragments of the broken third chromosome might go together with the attached fourth chromosome to the other pole (figures 1 and 3). Gametes 1 and 2 (figure 3) will be formed in this case. However, either of the frag-

ments of the broken third chromosome might go together with the unbroken third chromosome to the same pole of the spindle. As a result of such a distribution of the chromosome at the reduction division some of the gametes formed will carry a complete third chromosome plus a fragment of the other third chromosome (hyperploid gametes, figure 3, gametes 3 and 5). The other gametes will carry only one of the fragments of the third chromosome but will be deficient in respect to the other fragment of the same chromosome (hypoploid gametes, figure 3, gametes 4 and 6).

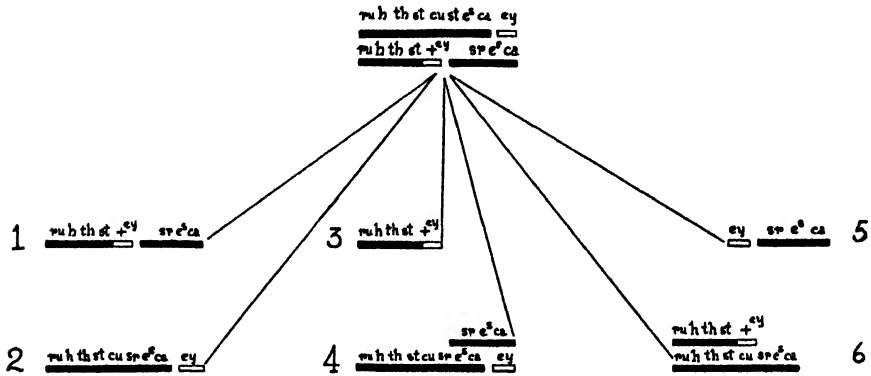


FIGURE 3.—Six kinds of gametes produced by a fly heterozygous for a translocation. Third chromosome—black; fourth chromosome—white.

The gametes 1 and 2 (figure 3) may be called “regular” gametes, whereas gametes 3 to 6 are “non-disjunctional” for one or the other section of the third chromosome. If a fly carrying a translocation is crossed to a fly free from translocation the regular gametes will give rise to zygotes carrying translocation and to normal zygotes respectively (figure 1). The non-disjunctional gametes will give rise to zygotes carrying a duplication or a deficiency for a section of the third chromosome. Flies developing from the latter zygotes must be expected to possess external peculiarities due to the genic unbalance. No such flies have been found in the cultures of any of the translocations here described. Hence, the non-disjunctional gametes are either not produced, or, more probably, the somatic changes in the zygotes, due to the changes in genic balance, are so great that the zygotes are unable to survive.

It is possible to test for the production of the non-disjunctional gametes by a genetical method. If two flies both carrying the same translocation are crossed to each other, gamete 3 from one parent united with gamete 4 from the other parent will give a normal zygote, since these gametes are com-

plementary to each other. Likewise, gamete 5 from one parent united with gamete 6 from the other parent must also give a normal zygote for the same reason. It is easy to make the zygotes produced by the union of the non-disjunctional gametes distinguishable in appearance from the zygotes produced by the union of the regular gametes. For this purpose it is necessary to introduce some recessive mutant genes into the chromosomes involved in the translocations.

Flies carrying translocations and at the same time homozygous in respect to various third-chromosome recessive genes may be easily obtained. In fact, many such flies have been observed during the course of the experiments planned for the purpose of studying the frequency of crossing over in the third chromosome (all the non-eyeless flies shown in tables 11-15 in the appendix). The appearance of such flies is due to crossing over between the fragments of the third chromosome involved in the translocations and the unbroken third chromosome carrying third-chromosome recessive genes. The following stocks of flies carrying translocations and homozygous with respect to certain third-chromosome recessive genes were established:

$$\begin{array}{ll}
 \text{a-translocation: } \frac{r_u \ h \ D}{r_u \ h} & \frac{s_r \ e^a \ c_a}{t_h \ s_t \ c_u \ s_r \ e^a \ c_a} \\
 \text{c-translocation: } \frac{r_u \ h \ t_h \ s_t}{r_u \ h \ t_h \ s_t} & \frac{s_r \ e^a \ c_a}{c_u \ s_r \ e^a \ c_a} \\
 \text{e-translocation: } \frac{r_u \ h \ t_h \ s_t}{r_u \ h \ t_h \ s_t} & \frac{s_r \ e^a \ c_a}{c_u \ s_r \ e^a \ c_a}
 \end{array}$$

Using these stocks and the regular stocks of the translocations carrying no third-chromosome recessive genes the following crosses were made:

$$\begin{array}{ll}
 \text{a-translocation: } \frac{r_u \ h \ D}{r_u \ h} & \frac{s_r \ e^a \ c_a}{t_h \ s_t \ c_u \ s_r \ e^a \ c_a} \quad \text{♀} \times \frac{D}{D} \quad \text{♂} \\
 \text{c-translocation: } \frac{r_u \ h \ t_h \ s_t}{r_u \ h \ t_h \ s_t} & \frac{s_r \ e^a \ c_a}{c_u \ s_r \ e^a \ c_a} \quad \text{♀} \times \frac{D}{D} \quad \text{♂} \\
 \text{e-translocation: } \frac{r_u \ h \ t_h \ s_t}{r_u \ h \ t_h \ s_t} & \frac{s_r \ e^a \ c_a}{c_u \ s_r \ e^a \ c_a} \quad \text{♀} \times \frac{D}{D} \quad \text{♂}
 \end{array}$$

If each of the parents in these crosses produces the six kinds of gametes shown in figure 3, thirty-six classes of zygotes must be produced (see table 7). In c- and e-translocations twenty-eight of these thirty-six classes must die since they carry either a deficiency or a duplication for a section of the third chromosome. The surviving classes are (table 7): Dichaete,

wild type, $D s_r e^+ c_a$ and $r_u h t_h s_t$. If the assortment of the fragments of the third chromosome in translocations takes place at random, all the six classes of gametes must be produced in equal numbers. Hence, the frequency of the surviving classes of zygotes must be: $3D$: 3 wild type: $1 D s_r e^+ c_a$: $1 r_u h t_h s_t$. If no non-disjunctive gametes are produced no $D s_r e^+ c_a$ and $r_u h t_h s_t$ individuals will appear. If, finally, the non-disjunctive gametes are produced less frequently than the regular gametes, the frequency of $D s_r e^+ c_a$ and $r_u h t_h s_t$ individuals will be lower than indicated above.

TABLE 7

	(1)	(2)	(3)	(4)	(5)	(6)
	r_u h t_h s_t	r_u h t_h s_t		r_u h t_h s_t		r_u h t_h s_t
	s_r e^+ c_a	s_r e^+ c_a	r_u h t_h s_t	s_r e^+ c_a	s_r e^+ c_a	s_r e^+ c_a
(1) $\frac{D}{\text{————}}$	<i>Dichaete</i>	<i>Dichaete</i>	dies	dies	dies	dies
(2) ————	<i>Wild type</i>	<i>Wild type</i>	dies	dies	dies	dies
(3) $\frac{D}{\text{————}}$	dies	dies	dies	$D s_r e^+ c_a$	dies	dies
(4) ————	dies	dies	<i>Wild type</i>	dies	dies	dies
(5) ————	dies	dies	dies	dies	dies	$r_u h t_h s_t$
(6) $\frac{D}{\text{————}}$	dies	dies	dies	dies	<i>Dichaete</i>	dies

The results actually obtained in experiments prove the last assumption, that is, they show that the non-disjunctive gametes are produced, but their frequency is much lower than the frequency of the regular gametes. Indeed, in e-translocation only 29 $r_u h t_h s_t$ and 2 $D s_r e^+ c_a$ individuals are observed among 672 flies (table 8). The appearance of the $r_u h t_h s_t$ individuals is due to non-disjunction of the left limb of the third chromosome, whereas the appearance of the $D s_r e^+ c_a$ individuals depends upon non-

disjunction of the right limb of the third chromosome (see figure 3 and table 7). In e-translocation the third chromosome is broken to the left of the spindle fibre, that is, the breakage point lies in the left limb of the third chromosome. The higher frequency of $r_u h t_h s_i$ individuals as compared with the $D s_r e^a c_a$ indicates that non-disjunction of the shorter fragment of the third chromosome (which is attached to the fourth chromosome) takes place more frequently than non-disjunction of the longer fragment of the third chromosome (which has retained its own spindle fibre).

In c-translocation the third chromosome is broken to the right of the spindle fibre. By analogy with e-translocation, non-disjunction of the right limb of the third chromosome must take place more frequently than the non-disjunction of the left limb in c-translocation. In other words, $D s_r e^a c_a$ individuals must be more frequent than $r_u h t_h s_i$ individuals. Indeed, 18 $D s_r e^a c_a$ and only 5 $r_u h t_h s_i$ individuals were observed in c-translocation (table 8).

TABLE 8
Non-disjunction of sections of the third chromosome in translocations.

Translocation a.					
$\frac{r_u h D}{r_u h} \frac{s_r e^a c_a}{t_h s_i c_u s_r e^a c_a} \text{ } \varnothing \times \frac{D}{\text{---}} \text{---} \text{ } \sigma^7$					
Class Number	Dichaete 196	Wild type 79	$r_u h D$ 31	$D s_r e^a c_a$ 2	Total 308
Translocation e.					
$\frac{r_u h t_h s_i}{r_u h t_h s_i} \frac{s_r e^a c_a}{c_u s_r e^a c_a} \text{ } \varnothing \times \frac{D}{\text{---}} \text{---} \text{ } \sigma^7$					
Class Number	Dichaete 305	Wild type 336	$r_u h t_h s_i$ 29	$D s_r e^a c_a$ 2	Total 672
Translocation c.					
$\frac{r_u h t_h s_i}{r_u h t_h s_i} \frac{s_r e^a c_a}{c_u s_r e^a c_a} \text{ } \varnothing \times \frac{D}{\text{---}} \text{---} \text{ } \sigma^7$					
Class Number	Dichaete 309	Wild type 374	$r_u h t_h s_i$ 5	$D s_r e^a c_a$ 18	Total 706

If two individuals both carrying a-translocation and carrying the third chromosomal genes indicated in table 8 are crossed to each other, twenty nine out of the thirty-six possible classes of offspring die. The remaining classes are: 4 $D : 1$ wild type: 1 $r_u h D : 1 D s_r e^a c_a$ (the indicated ratio holds true, of course, only in case all six possible classes of the gametes are produced in equal numbers). Actually the ratio 196 $D : 79$ wild type : 31 $r_u h D : 1 D s_r e^a c_a$ is observed (table 8). It follows that the non-

disjunctional classes of gametes are produced in a-translocation less frequently than regular gametes. In a-translocations the breakage of the third chromosome occurred at a point located slightly to the right of D , that is, in the left limb of the chromosome. Consequently the higher frequency of $r_u h D$ individuals as compared with $D s, e^+ c_a$ individuals indicates that non-disjunction of the fragment of the third chromosome attached to the fourth takes place more frequently than the non-disjunction of the other fragment of the third chromosome which has retained its own spindle fibre.

Taken as a whole, the results of the experiments just described are important in two respects. First, these results may be explained only on the assumption that the third chromosome in the translocations is broken into two fragments. In other words, the most important result of the cytological investigation of the translocations is proved by independent genetical evidence. Second, the experiments with non-disjunction of the sections of the third chromosome definitely prove that the different fragments of the third chromosome observed in the chromosomal plates of the translocations contain different blocks of genes corresponding to definite sections of the map of the third chromosome.

NON-DISJUNCTION OF THE FOURTH CHROMOSOMES IN THE TRANSLOCATIONS

Only one free fourth chromosome is seen in most of the chromosomal plates in the translocations. Since the flies carrying translocations are identical with normal flies in appearance and do not possess the characteristics of Haplo-IV, the presence of one more fourth chromosome in their cells is postulated. This fourth chromosome is evidently attached to one of the fragments of the third chromosome. The resulting compound appears in most of the translocations as an unpaired, rod-shaped chromosome in which no dividing line between the fourth chromosome and the fragment of the third is noticeable.

The conjugation of the fourth chromosomes in the translocations must take place in rather unusual conditions, since only one of these chromosomes is free, and the other is permanently attached to the fragment of the third chromosome. Nevertheless, as shown by the genetical behavior of the translocations, the reduction division proceeds more or less normally, and most of the gametes produced carry only one fourth chromosome. That is to say, the free fourth chromosome goes together with the unbroken third chromosome to one pole, and the attached fourth chromosome goes together with both fragments of the broken third chromosome to another pole of the spindle (figure 1). However, the cytological investigation revealed the occurrence of individuals having two free fourth chromosomes

in their cells. Such individuals are especially common in d-translocation, but they are also found in e-translocation. The appearance of such individuals may be explained on the assumption that the non-disjunction of the fourth chromosomes occurs rather frequently in the translocations. The free fourth chromosome might go sometimes together with the attached fourth chromosome and with both fragments of the third chromosome to the same pole of the spindle at the reduction division. Gametes carrying two fourth chromosomes may be produced in this way. Such gametes will give rise to zygotes having three fourth chromosomes, two of the latter being free and one attached to the third chromosome.

Individuals having three fourth chromosomes (triplo-IV) are so little different from normal individuals (diplo-IV) in appearance that their identification on the basis of external characters is impracticable. Nevertheless, the presence of a certain percentage of triplo-IV individuals in the stocks of the translocations may be proved by genetical methods. Two genetical methods were devised to distinguish the triplo-IV individuals from the diplo-IV ones in the translocations.

The first of these methods consists in crossing flies carrying translocations to those carrying the fourth-chromosome gene Minute-IV. Minute-IV is known to be dominant over one dose of the normal allelomorph but recessive to two doses of the normal allelomorph (SCHULTZ 1929). Hence, in the progeny of such a cross, the flies carrying translocations can be at the same time Minute-IV only if they have only two fourth chromosomes (one free and another attached to the third). If the flies carrying translocations have three fourth chromosomes (two free and one attached to the third chromosome), no characteristics of Minute-IV will be manifest.

The second method is based on the fact that in the flies from stocks of the translocations the fourth chromosome attached to the third carries the normal allelomorph of e_v , whereas the free fourth chromosome (or the free fourth chromosomes) carry e_v . It is clear that if such a fly is crossed to a wild-type fly, and its offspring are then crossed to a fly homozygous for e_v , no e_v individuals will appear in the next generation unless the initial fly was a triplo-IV individual.

Both methods just described were used for testing the flies from the stocks of the translocations. The number of the diplo-IV and the triplo-IV flies found is shown in the right-hand column in table 9. The number of the diplo-IV and triplo-IV flies found cytologically is presented in the middle column of table 9.

As seen from table 9, the triplo-IV flies are most frequent in the stock of d-translocation and comparatively rare in the other translocations. The agreement of the results received by the genetical and the cytological methods is satisfactory.

BEHAVIOR OF THE TRANSLOCATIONS IN HOMOZYGOUS FORM

In the preceding sections we have been dealing with the behavior of the heterozygous translocations. As shown by the cytological data, one of the third chromosomes in the heterozygous translocations is broken into two

TABLE 9
Number of diplo-IV and triplo-IV flies found in the stocks of the translocations.

	CYTOLOGICALLY		GENETICALLY	
	DIPLO-IV	TRIPLO-IV	DIPLO-IV	TRIPLO-IV
a-translocation	15	..	12	1
b-translocation	15	..	12	1
c-translocation	11	..	10	2
d-translocation	4	8	6	14
e-translocation	11	2	16	2

fragments, but the other third chromosome is completely normal. In homozygous translocations two pairs of the fragments of the third chromosome and no normal, unbroken third chromosome must be present. Since some interesting genetical phenomena may arise as a result of such a condition, experiments were planned to secure some of the translocations in homozygous form.

In d-translocation the section of the third chromosome lying to the right of the gene c_a is known to be broken off and attached to the fourth chromosome. Males carrying in the third chromosome involved in the translocation the genes r_u , h , t_h , s_t , c_u , s_r and e^s and carrying in the normal third chromosome the genes r_u , h , t_h , s_t , c_u , s_r , e^s and c_a (that is of the constitution $\frac{r_u h t_h s_t c_u s_r e^s}{r_u h t_h s_t c_u s_r e^s c_a}$) were crossed to regular (*i.e.*, carrying no translocation) females having in one of their third chromosomes the dominant gene Deformed (D_f), the recessive c_a and the crossing over suppressors $C_{III R}$ and $C_{III L}$. In the F_1 generation of this cross Deformed non-claret individuals were selected and inbred. These individuals are heterozygous for the translocation and have the constitution: $\frac{r_u h t_h s_t c_u s_r e^s}{C_{III L} D_f C_{III R} c_a}$. In the next generation (F_2) D_f and $r_u h t_h s_t c_u s_r e^s$ individuals may be expected to appear

in the ratio 2:1 (the gene D_f is lethal when homozygous). The $r_u h t_h s_t c_u s_r e^a$ individuals must be homozygous for the translocation. However, only D_f individuals were received in F_2 (about 1000 flies were examined). In other words, d-translocation is lethal when homozygous.

In e-translocation the third chromosome is broken between the loci of the genes s_t and p , and the section containing the genes from r_u to s_t is attached to the fourth chromosome (see figure 2). Males of the constitu-

tion $\frac{r_u h t_h s_t}{r_u h t_h s_t c_u s_r e^a c_a} \frac{s_r e^a c_a}{c_u s_r e^a c_a}$ were crossed to regular females carrying in one of their

third chromosomes the dominant genes D and S_b and the recessives s_t , c_u and e^a . In the F_1 generation the individuals manifesting the characters of D , s_t , S_b and e^a were selected and inbred. These individuals have the consti-

tution: $\frac{r_u h t_h s_t}{D s_t c_u S_b} \frac{s_r e^a c_a}{e^a}$. In the F_2 generation some $r_u h t_h s_t s_r e^a c_a$

individuals appeared; they must be homozygous for e-translocation.

Individuals homozygous for e-translocation are normal in appearance and have a good viability. However, the females have rudimentary ovaries resembling those of the *Drosophila melanogaster*—*D. simulans* hybrids (STURTEVANT 1920). Therefore, they are completely sterile when crossed either to males homozygous for e-translocation or to wild-type males. Males homozygous for e-translocation are fertile, though they produce markedly fewer offspring than the wild-type males or males heterozygous for e-translocation.

In c-translocation the third chromosome is broken between the genes p and c_u , and the section of the third chromosome containing the genes from c_u to c_a (see figure 2) is attached to the fourth chromosome. Males of the

constitution $\frac{r_u h t_h s_t}{r_u h t_h s_t c_u s_r e^a c_a} \frac{e^a c_a}{c_u s_r e^a c_a}$ were crossed to regular females carrying in

one of their third chromosomes the genes D , s_t , c_u , S_b and e^a . In the F_1 generation $D s_t S_b e^a$ individuals were selected and inbred. They have the

constitution $\frac{r_u h t_h s_t}{D s_t c_u S_b e^a} \frac{e^a c_a}{c_u S_b e^a}$. In the F_2 some $r_u h t_h s_t e^a c_a$ individuals

appeared; they are homozygous for c-translocation. Flies homozygous for c-translocation appear normal somatically, and are fertile in both sexes, though females produce markedly fewer offspring than do wild-type females or females heterozygous for c-translocation.

The behavior of the different translocations in homozygous condition is, therefore, diverse: d-translocation is lethal when homozygous, e-translocation survives but is sterile in one sex, and c-translocation survives and is

fertile in both sexes. (The behavior of a- and b-translocations in homozygous form was not studied because in both of them the third chromosome involved in the translocation carries the dominant gene *D* which is lethal when homozygous. The locus of *D* is so close to the point at which the breakage of the third chromosome occurred in these translocations that it is very difficult to obtain them without the gene *D*.)

The causes which may hinder the viability of the homozygous translocations are obscure. Theoretically, flies heterozygous as well as homozygous for the translocations must have the same genic balance that the normal flies have, and, therefore, must be viable, normal in appearance and in fertility. Indeed, most of the known translocations are normal in heterozygous condition, but, so far as known, only the c-translocation described here approaches the expectation when homozygous.

"Pale" translocation described by BRIDGES is lethal when homozygous (BRIDGES 1923, MORGAN, BRIDGES, STURTEVANT 1925). This fact has been explained by BRIDGES as due to a loss of a fragment of the translocated section during the process of transfer. In other words, the flies homozygous for "Pale" translocation are, according to BRIDGES, homozygous also for a deficiency of a small section of one of the chromosomes involved in the translocation. This explanation is supported by the fact that if the individuals heterozygous for "Pale" translocation are homozygous for the sex-linked gene eosin (an eosin-like eye-color), the resulting color of the eyes is somewhat diluted. Thus, "Pale" translocation has an abnormal genic balance even in heterozygous condition.

Three explanations of the lethal effect (and of the disturbance of the genic balance in general) produced by some of the translocations in homozygous condition may be suggested. First, a lethal or semilethal mutation might arise in one of the chromosomes involved in the translocation. Second, the breakage of the chromosome necessary to produce a translocation might cause some injury to the chromosome involved. For instance, some genes neighboring to the point at which the breakage occurred might be lost or destroyed (see BRIDGES' explanation). Third, it is possible to suppose that the breakage of a chromosome may occur only at a point which has been previously injured by some agent, for instance by the X-ray treatment. The data at present available give no evidence as to which of these explanations is true in the cases of d- and e-translocations.

Females homozygous for c-translocation were studied cytologically. All the chromosomal plates found (plate 2, figures 34-38) contain four pairs of chromosomes. Two pairs out of these four are rod-shaped; one of them is

markedly shorter than the other. Moreover, one pair is V-shaped, and one pair is J-shaped. The J-shaped chromosomes have a subterminal spindle-fibre attachment. Taken as a whole, the chromosomal complex in the homozygous c-translocation is more like that of *Drosophila immigrans* Sturt. (see METZ and MOSES 1923, figure 2 D) or that of *Drosophila melanica* Sturt. (see METZ and MOSES 1923, figure 2 E) than like that of normal *Drosophila melanogaster*.

Nevertheless, the interpretation of the cytological features found in the homozygous translocation is easy if the conditions found in heterozygous c-translocation are taken into account (see plate 1, figures 14–19). The longer pair of the rod-shaped chromosomes found in homozygous translocation are obviously the X-chromosomes. The pair of V-shaped chromosomes are the second chromosomes. The shorter pair of rod-shaped chromosomes and the pair of J-shaped chromosomes are the fragments of the third chromosome. No free fourth chromosome is found in any of the plates studied in homozygous translocation. Obviously, the shorter pair of rod shaped chromosomes consists of the fragments of the third chromosome attached to the fourth chromosomes. Indeed, since only one free fourth chromosome is present in the plates studied in heterozygous c-translocation, no free fourth chromosome must be expected in the chromosomal complex of the homozygous c-translocation.

This interpretation may be tested genetically. If the third chromosome is broken into two separate chromosomes (the rod-shaped and the J-shaped), the genes localized in one of them must show no linkage with the genes localized in the other. In other words, the third-chromosome linkage group of *Drosophila melanogaster* must be broken into two separate linkage groups in the homozygous translocation. The point of the breakage of the third chromosome is located in c-translocation to the right of the spindle fibre, between the genes peach and curled. Hence, the rod-shaped chromosome corresponds to the section of the genetical map of the third chromosome extending from peach to claret; the J-shaped chromosome corresponds to the section extending from scarlet to roughoid (figure 2). The sections just mentioned must behave in the homozygous translocation as two independent linkage groups.

Females homozygous for c-translocation and having the constitution $\frac{r_u \ h \ t_h \ s_t}{r_u \ h \ t_h \ s_t} \frac{e^+ \ c_a}{e^+ \ c_a}$ were crossed to males heterozygous for c-translocation and

having the constitution $\frac{D}{-}$. In the F_1 generation D males were selected and crossed to females homozygous for $r_u, h, t_h, s_t, c_u, s_r, e^+, c_a$ and free from

the translocation. These D -males must have the constitution $\frac{D}{r_u h t_h s_i e^+ c_a}$, that is, they are homozygous for the translocation but heterozygous for the third-chromosomal genes indicated. Should the third chromosomes in the homozygous translocation not be broken, only D and $r_u h t_h s_i e^+ c_i$ individuals would appear in the offspring of this cross. However, if the third-chromosome linkage group, following the breakage of the third chromosome, is also broken into two independent linkage groups, D , $r_u h t_h s_i e^+ c_a$, $r_u h t_h s_i$ and $D e^+ c_a$ individuals will appear in the ratio 1:1:1:1. The results obtained in the experiment are:

D	$r_u h t_h s_i$	$D e^+ c_a$	$r_u h t_h s_i e^+ c_a$	Total
232	243	238	227	940

Hence, two "new" linkage groups of genes replace, in the homozygous translocation, the original third-chromosome linkage group of *Drosophila melanogaster*. If females homozygous for c-translocation and having the constitution $\frac{D}{r_u h t_h s_i e^+ c_a}$ are crossed to homozygous $r_u h t_h s_i c_u s_r e^+ c_a$ males free from the translocation, the two "new" linkage groups remain independent from each other, but some crossing over occurs within each of them. The frequency of crossing over in the "new" linkage groups is shown in table 10 which is based on the counts of 752 flies.

TABLE 10

Frequency of crossing over in the "new" linkage groups in the homozygous translocation.

	INTERVAL	FREQUENCY IN HOMOZYGOUS TRANS- LOCATION—PERCENT	STANDARD FREQUENCY PERCENT	DIFFERENCE
The J-shaped chromo- some	r_u-h	27.8	26.5	+1.3
	$h-D$	14.4	13.9	+0.5
	$D-t_h$	1.2	1.8	-0.6
	t_h-s_i	1.5	1.8	-0.3
	s_i-e^+	46.5	26.7	+19.8
The rod-shaped chromosome	e^+-c_a	31.1	30.0	+1.1

It may be concluded that the frequencies of crossing over in the J-shaped chromosome are approximately equal to the frequencies of crossing over in the corresponding section of the normal, unbroken, third chromosome. The same is true in respect to the frequency of crossing over in the

e^+-c_a interval in the rod-shaped chromosome. Whether the frequency of crossing over in the whole rod-shaped chromosome is also equal to that in the corresponding section of the normal third chromosome, can not be decided on the basis of the data at hand. It may conceivably be different, since the rod-shaped chromosome in c-translocation contains not only the material corresponding to a section of the third chromosome, but also that corresponding to the fourth chromosome of the normal fly. The presence of the fourth-chromosome material in the rod-shaped chromosome might alter the frequency of crossing over in the neighboring part of the third chromosome as compared with the normal condition. This question needs further study.

In any case, the data presented here show that the classical assumption, according to which each linkage group corresponds to a separate chromosome, is correct.

DISCUSSION OF THE RESULTS

The cytological study of the translocations has shown that in each of them the third chromosome is broken into two fragments of unequal length and that the shorter fragment is attached to the fourth chromosome. The non-disjunction of the sections of the third chromosome in the translocations furnishes a genetical proof of the breakage of the third chromosomes. Finally, the results of the observations on the behavior of the homozygous translocation must be evaluated as the most convincing evidence in favor of our interpretation of the translocations.

The loci at which the breakage of the third chromosome took place in each of the translocations are determined by two completely independent methods. The first of these methods is a purely genetical one: the study of the linkage relations of the genes belonging to the third and to the fourth linkage groups. The location of the break is determined by this method in terms of units of map-distance, by essentially the same procedure as that by which the location of a mutant gene in the third chromosome of *Drosophila* is usually studied. The relative distances between the breaking-points in the different translocations may be compared in the same way in which the relative distances between the genes located in the third chromosome may be compared (figure 2). In doing so we proceed on the same basic assumption on which the genetical "map" of the third chromosome or the genetical map of any other chromosome is built. That is the assumption that the genes within a chromosome are arranged in linear order, and that the frequency of crossing over between any two genes

located in the same chromosome is a function of the distance between these genes.

The second method of determination of the locus at which the breakage of the chromosome took place is the direct investigation of the length of the fragments of the chromosome in cytological preparations. Since the shape of the chromosome during the metaphase stage is relatively constant, the location of the breakages may be expressed in terms of their distance from the ends of the chromosome, from the spindle fibre, from the constrictions, and from each other.

Assuming that the theory of linear arrangement of the genes within the chromosome is correct, the cytological conditions may be predicted on the basis of the knowledge of the genetical location of the breaking-point and vice versa. It has been shown in the preceding sections that such a prediction is really possible. In a- and b-translocations the loci of breakage as determined genetically nearly coincide with each other (figure 2). The chromosomes of a-translocation are nearly indistinguishable from those of b-translocation. In e-translocation the locus of the breakage on the basis of the genetical data must be supposed to lie between that observed in a- and b-translocations and the spindle fibre. This is exactly what has been found cytologically in e-translocation. The genetical data show that only a very small piece of the third chromosome may be attached to the fourth in d-translocation. Correspondingly, only a slight increase of one of the fourth chromosomes is observed cytologically. On the basis of the genetical evidence the third chromosome is broken in c-translocation at approximately the same distance from the spindle fibre as in e-translocation. The fragments of the third chromosome observed cytologically in c-translocation are but slightly different in size from those observed in e-translocation.

There is no escape from the conclusion that the sequence of the third-chromosomal genes as represented by the genetical map of this chromosome is the same as their sequence in the chromosome studied cytologically. In other words the genetical map represents the actual sequence of genes within the chromosome. Thus, the theory of the linear arrangement of the genes receives a strong basis of cytological evidence.

If the question about the reality of the linear order of genes within the chromosome is answered in the affirmative, it becomes possible to raise another question. Are the relative distances between the genes correctly represented by the genetical map of the third chromosome, or does the

genetical map give a somewhat distorted image of the actual spatial relations of the genes?

The cytologically observed points at which the third chromosome is broken in different translocations form a skeleton of a "cytological map" of the third chromosome. Furthermore, since certain genes are known to be adjacent to the points at which the breakage of the chromosome has occurred in different translocations, it seems to be justified to conclude that these genes are located in the chromosome in the neighborhood of the observed breakage-points. Thus, the "cytological map" represents the loci of the observed breakages, and, by inference, the loci of some of the third-chromosomal genes. The genetical and the cytological maps of the third chromosome, drawn to the same scale, are shown in figure 4.

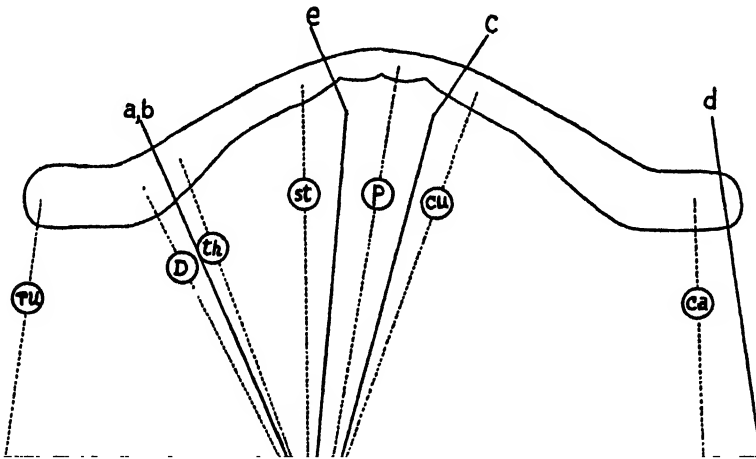


FIGURE 4.—Comparison of a genetical (below) and a cytological (above) map of the third chromosome of *Drosophila melanogaster*. a, b, c, d and e—the observed breaking-points. *r_w*—roughoid; *D*—Dichaete; *th*—thread; *st*—scarlet; *P*—peach; *cu*—curled; *ca*—claret.

It must be emphasized, that the cytological map presented in figure 4 is far less exact than the standard genetical map. First of all, the exact measurement of the fragments of the chromosomes of *Drosophila* is exceedingly difficult because of their extreme smallness. Furthermore, the presence of the fourth chromosome attached to one of the fragments of the third chromosome makes the estimation of the size of the latter still less exact. A certain alteration of the shape of the fragments of the third chromosome after breakage is also possible. Finally, the number of breakages of the third chromosome so far observed is too small to allow the construction of a detailed cytological map. Nevertheless, the relative distances

between the genes as represented by the genetical and the cytological map are so widely different, that certain conclusions may be drawn even on the basis of study of the cytological map available at present.

The distance between the locus of the breakage observed in a-translocation and the left-hand end of the third chromosome (gene *roughoid*) equals (at least) 41 map-units. The distance between the breakage observed in a-translocation and that observed in e-translocation equals only about 4 map-units. In other words, the distance between the left end of the third chromosome and the breakage observed in a-translocation is at least ten times longer than the distance between the breakages observed in a- and in e-translocations. That is the determination based on the genetical data. However, the cytological map shows that the latter distance is actually longer than the former. If studied genetically, the distance between the breakages observed in c- and c-translocations equals about 5 map-units, as compared with the 40 (or more) units distance between the breaking-point observed in a-translocation and the left-hand end of the third chromosome. Cytologically these distances are about equal in length. The distance between the point of the breakage observed in c-translocation and the right-hand end of the third chromosome is at least 60 units long; the distance between the points of breakage observed in e- and in c-translocations is about 5 units long. Cytologically, the former distance is only about twice as long as the latter (see figure 4).

PAINTER and MULLER (1929) have shown that the section of the third chromosome extending from sooty to the right-hand end of the third chromosome (this section must be at least 35 units long) is actually so small that it becomes scarcely visible if attached to the second chromosome.

Thus, the discrepancies between the length of the different sections of the third chromosome estimated on the basis of the genetical data and their length actually observed are very great. All these discrepancies may, however, be generalized in a form which discloses a very interesting regularity. The distances between the loci of the breakages or of the genes lying in the middle part of the chromosome are longer cytologically than they might be expected to be on the basis of the genetical map. On the other hand, the distances between the loci lying near the ends of the chromosome are shorter cytologically than they might be expected to be on the basis of the genetical data. The genetical map represents the genes located in the middle part of the chromosome (near the spindle fibre) relatively too close to each other, and the genes located at the ends of the chromosomes relatively too far apart.

In the metaphase stage the ends of the third chromosome appear to be considerably thicker than the middle part of the chromosome. This is partly due to a stronger condensation of the material located near the ends of the chromosome as compared with the material located near its middle, and partly to a premature splitting of the ends of the chromosome (compare figure 5, plate 1, representing the chromosome in the late prophase stage with the other figures representing chromosomes in the metaphase stage). This may suggest that the distances between the genes located near the ends of the chromosome are relatively shorter when the chromosome is in the metaphase stage than they are in the stage when crossing over takes place. However, the discrepancies between the relative distances of the genes from each other shown by the genetical and the cytological maps are too great to be accounted for by the different diameter of the different parts of the third chromosome.

The distances between the genes on the genetical map represent nothing but the frequency of crossing over between these genes. There is, however, no evidence that the frequency of crossing over between any two genes must be necessarily proportional to the absolute distance between these genes in the chromosome. In fact, there exists some evidence against such an assumption. The genes are not distributed at random along the genetical maps of the chromosomes but are crowded in some regions and sparse in others. This fact leads to the hypothesis according to which the frequency of crossing over per unit of the absolute distance may be different in different regions of the same chromosome (MORGAN, BRIDGES, STURTEVANT 1925, p. 93). The map of the third chromosome shows that the genes are crowded in the region neighboring to the spindle-fibre attachment but both ends of the map have few genes and long intervals in which no genes are known (figure 2).

If we suppose that the frequency of crossing over per unit of the absolute distance is much higher near the ends of the third chromosome than near the spindle fibre, the observed discrepancies between the genetical and the cytological maps of this chromosome find a simple explanation. The genetical map of the third chromosome represents correctly the actual sequence of the genes within the chromosome. However, the distances between the genes shown by the genetical map are functions of the actual distance between them in the chromosome as well as of the regional differences in the frequency of crossing over. In its turn, the frequency of crossing over per unit of the absolute distance in a given region seems to be a function of the distance between this region and the locus of the spindle-fibre attachment.

A similar conclusion is reached by PAINTER and MULLER (1929) on the basis of study of the deletions in the X-chromosome of *Drosophila melanogaster*. In this case the cytological data indicate that the actual distances between the genes located in the left-hand end of the X-chromosome are relatively longer than the distances between these genes suggested by the genetical map of this chromosome. The genetical map of the X-chromosome shows that the genes are strongly crowded at the left-hand end of the chromosome.

It is fairly certain on the basis of the data at hand that the genes are distributed at random along the genetical map of the third chromosome of *Drosophila melanogaster*, or at least much more nearly so than on the genetical map. Both the genetical and the cytological maps show only those genes which have given mutations. It follows that the process of mutation affects all the parts of the chromosome more or less at random.

SUMMARY

1. Five cases of translocation involving the third and the fourth chromosomes were observed in the progeny of flies treated by X-rays. In each of the five cases a section of the third chromosome became broken off and attached to the fourth chromosome.

2. Flies carrying translocations in heterozygous form are apparently normal in appearance and in viability, and nearly normal in fertility.

3. The loci at which the breakage of the third chromosome took place in each of the translocations were determined by studying the linkage between the genes of the third and those of the fourth chromosome linkage groups, which in the translocations behave as if they were localized in the same chromosome. The loci of breakage of the third chromosome are different in different translocations.

4. The frequency of crossing over is markedly decreased in the limb of the third chromosome in which the breakage has occurred, and slightly increased in the opposite limb. The point dividing the two limbs (the point of the spindle-fibre attachment) is slightly to the left of the locus of peach.

5. Non-disjunction of the sections of the third chromosome occurs in the translocations and can be detected by a suitable genetical method (see text). The study of the non-disjunction of the sections of the third chromosome gives a proof of the presence of the breakages of the third chromosome in the translocations.

6. Non-disjunction of the fourth chromosomes also occurs in the translocations.

7. The cytological investigation has shown that the longer pair of the V-shaped chromosomes is that affected by the translocations. It may be concluded that the longer pair of the V-shaped autosomes of *Drosophila melanogaster* carries the third-chromosome linkage group, and the shorter pair of the V-shaped autosomes carries the second-chromosome linkage group of the genes.

8. One of the third chromosomes is found cytologically to be broken into fragments in the translocations. One of the fragments is apparently attached to the fourth chromosome, since only one free fourth chromosome is present in most plates studied.

9. The length of the fragments of the third chromosome observed in cytological preparations is roughly proportional to their length suggested by the genetical data. This fact may be considered as a cytological proof of the theory of linear arrangement of the genes within the chromosomes.

10. A "cytological map" of the third chromosome is constructed on the basis of the cytological study of the translocations (see figure 4 in text). The comparison of the cytological map with the regular genetical map of the third chromosome shows that the distances between the genes located in the middle part of the chromosome are larger cytologically than they might be expected to be on the basis of the genetical map. This is apparently due to the low frequency of crossing over per unit of absolute distance in the middle part of the third chromosome, and to the high frequency of crossing over near the ends of the chromosome.

11. Two of the translocations were obtained in homozygous form. Individuals homozygous for the translocations are normal in appearance but sometimes sterile in the female.

12. Cytological study of the homozygous translocation showed, according to expectation, that both third chromosomes are broken into two fragments. No free fourth chromosome is found in homozygous translocation. Hence, the homozygous translocation possesses four pairs of chromosomes but two of them are very different in appearance from the chromosomes found in the normal fly.

13. The third-chromosome linkage group of *Drasophila melanogaster* is broken in homozygous translocation into two independent linkage groups of genes corresponding to the different sections of the normal third chromosome. This is, undoubtedly, due to the breakage of the third chromosome.

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APPENDIX—TABLES 11–17.

TABLE 11

Translocation a.

$$e_y \quad r_u \quad h \quad 2 \quad 3 \quad 4 \quad t_h \quad s_i \quad c_u \quad s_r \quad e^a \quad c_a$$

$$\dots \quad 1 \quad D + \quad 5 \quad 6 \quad 7 \quad 8 \quad 9 \quad \varphi \times e_y r_u h t_h s_i c_u s_r e^a c_a \sigma^r.$$

CLASSES OF THE OFFSPRING	NUMBER OBSERVED	CLASSES OF THE OFFSPRING	NUMBER OBSERVED
0 $\begin{cases} D \\ e_y r_u h t_h s_i c_u s_r e^a c_a \end{cases}$	508 143	8, 9 $\begin{cases} D e^a \\ e_y r_u h t_h s_i c_u s_r c_a \end{cases}$	17 3
Total non-crossovers	651	1, 8 $\begin{cases} r_u D e^a c_a \\ e_y h t_h s_i c_u s_r \end{cases}$	26 4
1 $\begin{cases} D r_u \\ e_y h t_h s_i c_u s_r e^a c_a \end{cases}$	140 33 24	2, 8 $\begin{cases} r_u h D e^a c_a \\ e_y t_h s_i c_u s_r \end{cases}$	2 1
2 $\begin{cases} D r_u h \\ e_y t_h s_i c_u s_r e^a c_a \end{cases}$	11	4, 8 $D t_h s_i c_u s_r$	1
3 $e_y D t_h s_i c_u s_r e^a c_a$	1	6, 8 $\begin{cases} D c_u s_r \\ e_y r_u h t_h s_i e^a c_a \end{cases}$	9 4
4 $e_y r_u h$	1	1, 7 $\begin{cases} r_u D s_r e^a c_a \\ e_y h t_h s_i c_u \end{cases}$	22 14
5 $\begin{cases} D s_i c_u s_r e^a c_a \\ e_y r_u h t_h \end{cases}$	1 2	2, 7 $\begin{cases} r_u h D s_r e^a c_a \\ e_y t_h s_i c_u \end{cases}$	4 4
6 $\begin{cases} D c_u s_r e^a c_a \\ e_y r_u h t_h s_i \end{cases}$	55 25	6, 7 $\begin{cases} D c_u \\ e_y r_u h t_h s_i s_r e^a c_a \end{cases}$	2 3
7 $\begin{cases} D s_r e^a c_a \\ e_y r_u h t_h s_i c_u \end{cases}$	156 56	5, 7 $e_y r_u h t_h s_r e^a c^a$	2
8 $\begin{cases} D e^a c_a \\ e_y r_u h t_h s_i c_u s_r \end{cases}$	120 54	1, 6 $\begin{cases} r_u D c_u s_r e^a c_a \\ e_y h t_h s_i \end{cases}$	5 1
9 $\begin{cases} D c_a \\ e_y r_u h t_h s_i c_u s_r e^a \end{cases}$	318 83	2, 6 $r_u h D c_u s_r e^a c_a$	1
Total single-crossovers	1080	Total double crossovers	332
1, 9 $\begin{cases} r_u D c_a \\ e_y h t_h s_i c_u s_r e^a \end{cases}$	82 13	1, 7, 9 $\begin{cases} r_u D s_r e^a \\ e_y h t_h s_i c_u c_a \end{cases}$	8 4
2, 9 $\begin{cases} r_u h D c_a \\ e_y t_h s_i c_u s_r e^a \end{cases}$	7 7	1, 6, 9 $\begin{cases} r_u D c_u s_r e^a \\ e_y h t_h s_i c_a \end{cases}$	3 1
6, 9 $\begin{cases} D c_u s_r e^a \\ e_y r_u h t_h s_i c_u \end{cases}$	12 15	1, 6, 8 $r_u D c_u s_r$	1
7, 9 $\begin{cases} D s_r e^a \\ e_y r_u h t_h s_i c_u c_a \end{cases}$	52 19	1, 8, 9 $r_u D e^a$	1
		Total triple crossovers	18
		Grand total	2081

TABLE 12
Translocation b.

$$\begin{array}{cccccccccc} \phi_y & r_u & h & 2 & 3 & 4 & t_h & s_i & c_u & s_r & e^o & c_a \\ \hline \dots & 1 & D & + & 5 & 6 & 7 & 8 & 9 & & & \end{array} \quad \phi \times \phi_y r_u h t_h s_i c_u s_r e^o c_a \sigma^o.$$

CLASSES OF THE OFFSPRING	NUMBER OBSERVED	CLASSES OF THE OFFSPRING	NUMBER OBSERVED
0 $\left\{ \begin{array}{l} D \\ e_y r_u h t_h s_i c_u s_r e^o c_a \end{array} \right.$	$\begin{array}{l} 304 \\ 110 \end{array}$	6, 9 $\left\{ \begin{array}{l} D c_u s_r e^o \\ e_y r_u h t_h s_i c_a \end{array} \right.$	$\begin{array}{l} 7 \\ 9 \end{array}$
Total non-crossovers	414		
1 $\left\{ \begin{array}{l} r_u D \\ \phi_y h t_h s_i c_u s_r e^o c_a \\ r_u h D \end{array} \right.$	$\begin{array}{l} 129 \\ 22 \\ 27 \end{array}$	7, 9 $\left\{ \begin{array}{l} D s_r e^o \\ e_y r_u h t_h s_i c_u c_a \\ D e^o \end{array} \right.$	$\begin{array}{l} 22 \\ 12 \\ 9 \end{array}$
2 $\left\{ \begin{array}{l} \phi_y t_h s_i c_u s_r e^o c_a \\ r_u h \end{array} \right.$	$\begin{array}{l} 18 \\ 1 \end{array}$	8, 9 $\left\{ \begin{array}{l} e_y r_u h t_h s_i c_u s_r c_a \\ r_u D e^o c_a \end{array} \right.$	$\begin{array}{l} 1 \\ 7 \end{array}$
3 $\left\{ \begin{array}{l} e_y D t_h s_i c_u s_r e^o c_a \\ D s_i c_u s_r e^o c_a \end{array} \right.$	$\begin{array}{l} 1 \\ 1 \end{array}$	1, 8 $\left\{ \begin{array}{l} e_y h t_h s_i c_u s_r \\ r_u h D e^o c_a \end{array} \right.$	$\begin{array}{l} 4 \\ 5 \end{array}$
5 $\left\{ \begin{array}{l} e_y r_u h t_h \\ D c_u s_r e^o c_a \end{array} \right.$	$\begin{array}{l} 4 \\ 20 \end{array}$	2, 8 $\left\{ \begin{array}{l} e_y t_h s_i c_u s_r \\ D c_u s_r \end{array} \right.$	$\begin{array}{l} 1 \\ 5 \end{array}$
6 $\left\{ \begin{array}{l} e_y r_u h t_h s_i \\ D s_r e^o c_a \end{array} \right.$	$\begin{array}{l} 17 \\ 114 \end{array}$	6, 8 $\left\{ \begin{array}{l} e_y r_u h t_h s_i e^o c_a \\ D r_u s_r e^o c_a \end{array} \right.$	$\begin{array}{l} 3 \\ 36 \end{array}$
7 $\left\{ \begin{array}{l} e_y r_u h t_h s_i c_u \\ D e^o c_a \end{array} \right.$	$\begin{array}{l} 35 \\ 69 \end{array}$	1, 7 $\left\{ \begin{array}{l} e_y h t_h s_i c_u \\ r_u h D s_r e^o c_a \end{array} \right.$	$\begin{array}{l} 10 \\ 8 \end{array}$
8 $\left\{ \begin{array}{l} e_y r_u h t_h s_i c_u s_r \\ D c_a \end{array} \right.$	$\begin{array}{l} 37 \\ 202 \end{array}$	2, 7 $\left\{ \begin{array}{l} e_y t_h s_i c_u \\ r_u D c_u s_r e^o c_a \end{array} \right.$	$\begin{array}{l} 3 \\ 5 \end{array}$
9 $\left\{ \begin{array}{l} e_y r_u h t_h s_i c_u s_r e^o \end{array} \right.$	71	1, 6 $r_u D c_u s_r e^o c_a$	1
		2, 6 $r_u h D c_u s_r e^o c_a$	1
		1, 2 $h D$	1
Total single crossovers	768	Total double crossovers	255
1, 9 $\left\{ \begin{array}{l} r_u D c_a \\ e_y h t_h s_i c_u s_r e^o \\ r_u h D c_a \end{array} \right.$	$\begin{array}{l} 45 \\ 40 \\ 16 \end{array}$	1, 7, 9 $r_u D s_r e^o$	5
2, 9 $\left\{ \begin{array}{l} e_y t_h s_i c_u s_r e^o \end{array} \right.$	4	2, 7, 9 $r_u h D s_r e^o$	1
3, 9 $r_u h c_a$	1	1, 8, 9 $r_u D e^o$	2
		7, 8, 9 $e_y r_u h t_h s_i c_u e^o$	1
		1, 2, 7 $e_y r_u t_h s_i c_u$	1
		Total triple crossovers	10
		Grand total	1447

TABLE 13
Translocation c.
 $e_y \ r_u \ h \ 2 \ 3 \ t_h \ s_i \ 5 \ 6 \ c_u \ s_r \ e^+ \ c_a$
 $\dots \ 1 \ D \ 4 \ + \ 7 \ 8 \ 9 \ \text{♀} \times e_y \ r_u \ h \ t_h \ s_i \ c_u \ s_r \ e^+ \ c_a \ \text{♂}.$

CLASSES OF THE OFFSPRING	NUMBER OBSERVED	CLASSES OF THE OFFSPRING	NUMBER OBSERVED
0 $\left\{ \begin{array}{l} D \\ e_y \ r_u \ h \ t_h \ s_i \ c_u \ s_r \ e^+ \ c_a \end{array} \right.$	675 240	7, 9 $\left\{ \begin{array}{l} D \ s_r \ e^+ \\ e_y \ r_u \ h \ t_h \ s_i \ c_u \ c_a \\ D \ e^+ \end{array} \right.$	8 4 6
Total non-crossovers	915	8, 9 $\left\{ \begin{array}{l} e_y \ r_u \ h \ t_h \ s_i \ c_u \ s_r \ c_a \\ r_u \ D \ e^+ \ c_a \end{array} \right.$	1 40
1 $\left\{ \begin{array}{l} r_u \ D \\ e_y \ h \ t_h \ s_i \ c_u \ s_r \ e^+ \ c_a \\ r_u \ h \ D \end{array} \right.$	396 143 198	1, 8 $\left\{ \begin{array}{l} e_y \ h \ t_h \ s_i \ c_u \ s_r \\ r_u \ h \ D \ e^+ \ c_a \end{array} \right.$	6 16
2 $\left\{ \begin{array}{l} e_y \ t_h \ s_i \ c_u \ s_r \ e^+ \ c_a \\ r_u \ h \end{array} \right.$	116 18	2, 8 $\left\{ \begin{array}{l} e_y \ t_h \ s_i \ c_u \ s_r \\ r_u \ h \ e^+ \ c_a \end{array} \right.$	11 3
3 $\left\{ \begin{array}{l} e_y \ D \ t_h \ s_i \ c_u \ s_r \ e^+ \ c_a \\ r_u \ h \ t_h \end{array} \right.$	5 11	3, 8 $\left\{ \begin{array}{l} r_u \ h \ e^+ \ c_a \\ r_u \ h \ t_h \ e^+ \ c_a \end{array} \right.$	1 2
4 $\left\{ \begin{array}{l} e_y \ D \ s_i \ c_u \ s_r \ e^+ \ c_a \\ r_u \ h \ t_h \ s_i \end{array} \right.$	11 37	4, 8 $\left\{ \begin{array}{l} e_y \ D \ c_u \ s_r \\ r_u \ h \ t_h \ s_i \ e^+ \ c_a \end{array} \right.$	4 1
5 $\left\{ \begin{array}{l} e_y \ D \ c_u \ s_r \ e^+ \ c_a \\ e_y \ r_u \ h \ t_h \ s_i \end{array} \right.$	26 8	5, 8 $\left\{ \begin{array}{l} r_u \ h \ t_h \ s_i \ e^+ \ c_a \\ D \ c_u \ s_r \end{array} \right.$	4 1
6 $\left\{ \begin{array}{l} D \ c_u \ s_r \ e^+ \ c_a \\ e_y \ r_u \ h \ t_h \ s_i \ c_u \end{array} \right.$	12 12	6, 8 $\left\{ \begin{array}{l} r_u \ D \ s_r \ e^+ \ c_a \\ e_y \ h \ t_h \ s_i \ c_u \\ r_u \ h \ D \ s_r \ e^+ \ c_a \end{array} \right.$	22 1 10
7 $\left\{ \begin{array}{l} D \ s_r \ e^+ \ c_a \\ e_y \ r_u \ h \ t_h \ s_i \ c_u \ s_r \end{array} \right.$	50 51	1, 7 $\left\{ \begin{array}{l} e_y \ h \ t_h \ s_i \ c_u \\ r_u \ h \ D \ s_r \ e^+ \ c_a \end{array} \right.$	2 2
8 $\left\{ \begin{array}{l} D \ e^+ \ c_a \\ e_y \ r_u \ h \ t_h \ s_i \ c_u \ s_r \ e^+ \end{array} \right.$	96 133	2, 7 $\left\{ \begin{array}{l} e_y \ t_h \ s_i \ c_u \\ r_u \ h \ s_r \ e^+ \ c_a \\ r_u \ h \ t_h \ s_r \ e^+ \ c_a \end{array} \right.$	3 4 4
9 $\left\{ \begin{array}{l} D \ c_a \\ e_y \ r_u \ h \ t_h \ s_i \ c_u \ s_r \ e^+ \end{array} \right.$	384 1	1, 5 $\left\{ \begin{array}{l} h \ t_h \ s_i \\ e_y \ r_u \ h \ D \ c_u \ s_r \ e^+ \ c_a \end{array} \right.$	10 1
Total single crossovers	1707	2, 5 $\left\{ \begin{array}{l} t_h \ s_i \\ D \ t_h \ s_i \\ h \ D \end{array} \right.$	5 2 9
1, 9 $\left\{ \begin{array}{l} r_u \ D \ c_a \\ e_y \ h \ t_h \ s_i \ c_u \ s_r \ e^+ \\ r_u \ h \ D \ c_a \end{array} \right.$	169 48 79	1, 2 $\left\{ \begin{array}{l} e_y \ r_u \ t_h \ s_i \ c_u \ s_r \ e^+ \ c_a \end{array} \right.$	1
2, 9 $\left\{ \begin{array}{l} e_y \ t_h \ s_i \ c_u \ s_r \ e^+ \\ r_u \ h \ c_a \end{array} \right.$	35 15	Total double crossovers	553
3, 9 $\left\{ \begin{array}{l} e_y \ D \ t_h \ s_i \ c_u \ s_r \ e^+ \\ r_u \ h \ t_h \ c_a \end{array} \right.$	2 2	1, 2, 9 $\left\{ \begin{array}{l} h \ D \ c_a \\ r_u \ h \ D \ s_r \ e^+ \end{array} \right.$	3 1
4, 9 $\left\{ \begin{array}{l} e_y \ D \ c_u \ s_r \ e^+ \\ r_u \ h \ t_h \ s_i \ c_a \end{array} \right.$	4 23	1, 7, 9 $\left\{ \begin{array}{l} r_u \ D \ s_r \ e^+ \\ h \ t_h \ s_i \ c_a \end{array} \right.$	1 2
5, 9 $\left\{ \begin{array}{l} r_u \ h \ t_h \ s_i \ c_a \\ D \ c_u \ s_r \ e^+ \end{array} \right.$	1 1	1, 5, 9 $\left\{ \begin{array}{l} h \ t_h \ s_i \ c_a \\ t_h \ s_i \ c_a \end{array} \right.$	1 1
6, 9 $\left\{ \begin{array}{l} r_u \ h \ t_h \ s_i \ c_a \\ D \ c_u \ s_r \ e^+ \end{array} \right.$	1 1	2, 5, 9 $\left\{ \begin{array}{l} t_h \ s_i \ c_a \\ r_u \ h \ D \ c_u \ s_r \end{array} \right.$	1 1
		1, 5, 7 $\left\{ \begin{array}{l} h \ t_h \ s_i \ s_r \ e^+ \ c_a \end{array} \right.$	1
		Total triple crossovers	10
		Grand total	3185

TABLE 14
Translocation d.

$$e_y \ r_u \ h \ 2 \ 3 \ t_h \ s_i \ c_u \ s_r \ e^+ \ c_a \ 9$$

$$\dots \quad 1 \quad D \quad 4 \quad 5 \quad 6 \quad 7 \quad 8 \quad +$$

$$\varphi \times e_y \ r_u \ h \ t_h \ s_i \ c_u \ s_r \ e^+ \ c_a \ \sigma^7.$$

CLASSES OF THE OFFSPRING	NUMBER OBSERVED	CLASSES OF THE OFFSPRING	NUMBER OBSERVED
0 $\left\{ \begin{array}{l} D \\ e_y \ r_u \ h \ t_h \ s_i \ c_u \ s_r \ e^+ \ c_a \end{array} \right.$	289 152	5, 8 $e_y \ r_u \ h \ t_h \ s_i \ c_a$ 6, 8 $D \ s_r \ e^+$	3 7
Total non-crossovers	441	7, 8 $D \ e^+$	1
1 $\left\{ \begin{array}{l} D \ r_u \\ e_y \ h \ t_h \ s_i \ c_u \ s_r \ e^+ \ c_a \end{array} \right.$	185 99	1, 7 $\left\{ \begin{array}{l} e_y \ r_u \ D \ e^+ \ c_a \\ h \ t_h \ s_i \ c_u \ s_r \end{array} \right.$	32 11
2 $\left\{ \begin{array}{l} D \ r_u \ h \\ e_y \ t_h \ s_i \ c_u \ s_r \ e^+ \ c_a \end{array} \right.$	77 81	2, 7 $\left\{ \begin{array}{l} e_y \ r_u \ h \ D \ e^+ \ c_a \\ t_h \ s_i \ c_u \ s_r \end{array} \right.$	10 4
3 $\left\{ \begin{array}{l} r_u \ h \\ e_y \ D \ t_h \ s_i \ c_u \ s_r \ e^+ \ c_a \end{array} \right.$	6 5	5, 7 $\left\{ \begin{array}{l} D \ c_u \ s_r \\ e_y \ r_u \ h \ t_h \ s_i \ e^+ \ c_a \end{array} \right.$	2 3
4 $\left\{ \begin{array}{l} r_u \ h \ t_h \\ e_y \ D \ s_i \ c_u \ s_r \ e^+ \ c_a \end{array} \right.$	3 7	1, 6 $\left\{ \begin{array}{l} h \ t_h \ s_i \ c_u \\ e_y \ r_u \ D \ s_r \ e^+ \ c_a \end{array} \right.$	14 38
5 $\left\{ \begin{array}{l} r_u \ h \ t_h \ s_i \\ e_y \ D \ c_u \ s_r \ e^+ \ c_a \end{array} \right.$	29 28	2, 6 $\left\{ \begin{array}{l} t_h \ s_i \ c_u \\ e_y \ r_u \ h \ D \ s_r \ e^+ \ c_a \end{array} \right.$	13 14
6 $\left\{ \begin{array}{l} r_u \ h \ t_h \ s_i \ c_u \\ e_y \ D \ s_r \ e^+ \ c_a \end{array} \right.$	41 72	3, 6 $\left\{ \begin{array}{l} D \ t_h \ s_i \ c_u \\ e_y \ r_u \ h \ s_r \ e^+ \ c_a \end{array} \right.$	1 3
7 $\left\{ \begin{array}{l} r_u \ h \ t_h \ s_i \ c_u \ s_r \\ e_y \ D \ e^+ \ c_a \end{array} \right.$	37 48	4, 6 $e_y \ r_u \ h \ t_h \ s_r \ e^+ \ c_a$	1
8 $\left\{ \begin{array}{l} r_u \ h \ t_h \ s_i \ c_u \ s_r \ e^+ \\ e_y \ D \ c_a \end{array} \right.$	82 79	5, 6 $e_y \ r_u \ h \ t_h \ s_i \ s_r \ e^+ \ c_a$	1
9 $\left\{ \begin{array}{l} r_u \ h \ t_h \ s_i \ c_u \ s_r \ e^+ \ c_a \\ e_y \ D \end{array} \right.$	8 21	1, 5 $\left\{ \begin{array}{l} h \ t_h \ s_i \\ e_y \ r_u \ D \ c_u \ s_r \ e^+ \ c_a \end{array} \right.$	6 6
Total single crossovers	908	2, 5 $\left\{ \begin{array}{l} t_h \ s_i \\ e_y \ r_u \ h \ D \ c_u \ s_r \ e^+ \ c_a \end{array} \right.$	4 1
1, 9 $\left\{ \begin{array}{l} e_y \ r_u \ D \\ h \ t_h \ s_i \ c_u \ s_r \ e^+ \ c_a \end{array} \right.$	17 4	3, 5 $D \ t_h \ s_i$	1
2, 9 $\left\{ \begin{array}{l} e_y \ r_u \ h \ D \\ t_h \ s_i \ c_u \ s_r \ e^+ \ c_a \end{array} \right.$	6 1	1, 2 $h \ D$	1
4, 9 $D \ s_i \ c_u \ s_r \ e^+ \ c_a$	1	Total double crossovers	367
5, 9 $\left\{ \begin{array}{l} D \ c_u \ s_r \ e^+ \ c_a \\ e_y \ r_u \ h \ t_h \ s_i \end{array} \right.$	2 1	1, 8, 9 $e_y \ h \ t_h \ s_i \ c_u \ s_r \ e^+$	1
6, 9 $D \ s_r \ e^+ \ c_a$	8	1, 5, 9 $e_y \ h \ t_h \ s_i$	1
8, 9 $\left\{ \begin{array}{l} D \ c_a \\ e_y \ r_u \ h \ t_h \ s_i \ c_u \ s_r \ e^+ \end{array} \right.$	5 9	5, 8, 9 $e_y \ D \ c_u \ s_r \ e^+$	1
1, 8 $\left\{ \begin{array}{l} h \ t_h \ s_i \ c_u \ s_r \ e^+ \\ e_y \ r_u \ D \ c_a \end{array} \right.$	31 51	7, 8, 9 $e_y \ D \ e^+$	1
2, 8 $\left\{ \begin{array}{l} t_h \ s_i \ c_u \ s_r \ e^+ \\ e_y \ r_u \ h \ D \ c_a \end{array} \right.$	26 13	1, 2, 8 $r_u \ t_h \ s_i \ c_u \ s_r \ e^+$	1
3, 8 $\left\{ \begin{array}{l} D \ t_h \ s_i \ c_u \ s_r \ e^+ \\ e_y \ r_u \ h \ c_a \end{array} \right.$	2 1	1, 6, 8 $r_u \ D \ s_r \ e^+$	1
4, 8 $D \ s_i \ c_u \ s_r \ e^+$	4	2, 6, 8 $r_u \ h \ D \ s_r \ e^+$	4
5, 8 $D \ c_u \ s_r \ e^+$	8	1, 2, 7 $r_u \ t_h \ s_i \ c_u \ s_r$	1
		1, 5, 7 $\left\{ \begin{array}{l} r_u \ D \ c_u \ s_r \\ e_y \ h \ t_h \ s_i \ e^+ \ c_a \end{array} \right.$	3 1
		2, 5, 7 $r_u \ h \ D \ s_r$	1
		Total triple crossovers	16
		1, 5, 8, 9 $h \ t_h \ s_i \ c_a$	1
		Total quadruple crossovers	1
		Grand total	1733

TABLE 15
Translocation *e*.
$$e_y \quad r_u \quad h \quad 2 \quad 3 \quad t_h \quad s_i \quad 5 \quad 6 \quad c_u \quad s_r \quad e^+ \quad c_a$$

$$\dots \quad 1 \quad D \quad 4 \quad + \quad 7 \quad 8 \quad 9$$

$$Q \times e_y r_u h t_h s_i c_u s_i e^+ c_a \sigma^7.$$

CLASSES OF THE OFFSPRING	NUMBER OBSERVED	CLASSES OF THE OFFSPRING	NUMBER OBSERVED
0 $\left\{ \begin{array}{l} D \\ e_y r_u h t_h s_i c_u s_r e^+ c_a \end{array} \right.$	285 81	7, 9 $\left\{ \begin{array}{l} D s_r e^+ \\ e_y r_u h t_h s_i s_u c_a \end{array} \right.$	26 6
Total non-crossovers	366	8, 9 $\left\{ \begin{array}{l} D e^+ \\ e_y r_u h t_h s_i c_u s_r c_a \end{array} \right.$	5 5
1 $\left\{ \begin{array}{l} r_u D \\ e_y h t_h s_i c_u s_r e^+ c_a \end{array} \right.$	135 24	1, 8 $\left\{ \begin{array}{l} r_u D e^+ c_a \\ r_u h D e^+ c_a \end{array} \right.$	17 10
2 $\left\{ \begin{array}{l} r_u h D \\ e_y t_h s_i c_u s_r e^+ c_a \end{array} \right.$	49 43	3, 8 $\left\{ \begin{array}{l} r_u h e^+ c_a \\ r_u h t_h s_i e^+ c_a \end{array} \right.$	6 6
3 $\left\{ \begin{array}{l} e_y D t_h s_i c_u s_r e^+ c_a \\ r_u h \end{array} \right.$	1 6	5, 8 $\left\{ \begin{array}{l} D c_u s_r \\ e_y r_u h t_h s_i e^+ c_a \end{array} \right.$	3 1
4 $\left\{ \begin{array}{l} e_y D s_i c_u s_r e^+ c_a \\ r_u h t_h \end{array} \right.$	2 2	1, 7 $\left\{ \begin{array}{l} r_u D s_r e^+ c_a \\ e_y h t_h s_i c_u \end{array} \right.$	20 10
5 $\left\{ \begin{array}{l} e_y D c_u s_r e^+ c_a \\ r_u h t_h s_i \end{array} \right.$	6 6	$\left\{ \begin{array}{l} r_u h D s_r e^+ c_a \\ e_y t_h s_i c_u \end{array} \right.$	11 7
6 $\left\{ \begin{array}{l} D c_u s_r e^+ c_a \\ e_y r_u h t_h s_i \end{array} \right.$	20 14	2, 7 $\left\{ \begin{array}{l} r_u h s_r e^+ c_a \\ r_u h t_h s_r e^+ c_a \end{array} \right.$	1 1
7 $\left\{ \begin{array}{l} D s_r e^+ c_a \\ e_y r_u h t_h s_i c_u \end{array} \right.$	117 27	4, 7 $\left\{ \begin{array}{l} r_u h t_h s_r e^+ c_a \\ r_u h D c_u s_r e^+ c_a \end{array} \right.$	1 2
8 $\left\{ \begin{array}{l} D e^+ c_a \\ e_y r_u h t_h s_i c_u s_r \end{array} \right.$	85 33	2, 5 $\left\{ \begin{array}{l} t_h s_i \end{array} \right.$	1
9 $\left\{ \begin{array}{l} D c_a \\ e_y r_u h t_h s_i c_u s_r e^+ \end{array} \right.$	199 67	Total double crossovers	282
Total single crossovers	836	1, 8, 9 $\left\{ \begin{array}{l} r_u D e^+ \end{array} \right.$	1
1, 9 $\left\{ \begin{array}{l} r_u D c_a \\ e_y h t_h s_i c_u s_r e^+ \end{array} \right.$	57 24	1, 7, 9 $\left\{ \begin{array}{l} r_u D s_r e^+ \end{array} \right.$	1
2, 9 $\left\{ \begin{array}{l} r_u h D c_a \\ e_y t_h s_i c_u s_r e^+ \end{array} \right.$	39 7	1, 6, 9 $\left\{ \begin{array}{l} e_y h t_h s_i c_a \end{array} \right.$	1
3, 9 $\left\{ \begin{array}{l} r_u h c_a \\ e_y D t_h s_i c_u s_r e^+ \end{array} \right.$	1 1	5, 7, 9 $\left\{ \begin{array}{l} r_u h t_h s_i s_r e^+ \end{array} \right.$	2
4, 9 $\left\{ \begin{array}{l} e_y D s_i c_u s_r e^+ \\ r_u h t_h c_a \end{array} \right.$	1 1	2, 8, 9 $\left\{ \begin{array}{l} e_y t_h s_i c_u s_r c_a \end{array} \right.$	1
5, 9 $\left\{ \begin{array}{l} r_u h t_h s_i c_a \\ e_y r_u h t_h s_i c_a \end{array} \right.$	1 1	1, 2, 9 $\left\{ \begin{array}{l} e_y r_u t_h s_i c_u s_r e^+ \end{array} \right.$	1
6, 9 $\left\{ \begin{array}{l} D c_u s_r e^+ \end{array} \right.$	7 5	6, 7, 9 $\left\{ \begin{array}{l} e_y r_u h t_h s_i s_r e^+ \end{array} \right.$	1
		Total triple-crossovers	8
		1, 2, $\left\{ \begin{array}{l} e_y r_u t_h s_i c_u c_a \end{array} \right.$	1
		7, 9 $\left\{ \begin{array}{l} h D s_r e^+ \end{array} \right.$	3
		Total quadruple crossovers	4
		Grand total	1496

TABLE 16
Crossing over in the normal third chromosome—control experiment.

$e_y \quad r_u \quad h \quad 2 \quad 3 \quad t_h \quad s_t \quad c_u \quad s_r \quad e^+ \quad c_a$		$\varphi \times e_y \quad r_u \quad h \quad t_h \quad s_t \quad c_u \quad s_r \quad e^+ \quad c_a \quad \sigma^7$	
+		1 D 4 5 6 7 8	
CLASSES OF THE OFFSPRING	NUMBER OBSERVED	CLASSES OF THE OFFSPRING	NUMBER OBSERVED
0 $\begin{cases} D \\ r_u \quad h \quad t_h \quad s_t \quad c_u \quad s_r \quad e^+ \quad c_a \end{cases}$	$\begin{matrix} 354 \\ 165 \end{matrix}$	4, 7 $\begin{cases} D \quad s_t \quad c_u \quad s_r \\ D \quad c_u \quad s_r \end{cases}$	$\begin{matrix} 1 \\ 1 \end{matrix}$
Total non-crossovers	519	5, 7 $\begin{cases} r_u \quad h \quad t_h \quad s_t \quad e^+ \quad c_a \\ D \quad s_r \end{cases}$	$\begin{matrix} 10 \\ 1 \end{matrix}$
1 $\begin{cases} r_u \quad D \\ h \quad t_h \quad s_t \quad c_u \quad s_r \quad e^+ \quad c_a \end{cases}$	$\begin{matrix} 144 \\ 56 \end{matrix}$	7, 6 $\begin{cases} r_u \quad h \quad t_h \quad s_t \quad c_u \quad e^+ \quad c_a \\ r_u \quad D \quad s_r \quad e^+ \quad c_a \end{cases}$	$\begin{matrix} 6 \\ 41 \end{matrix}$
2 $\begin{cases} r_u \quad h \quad D \\ t_h \quad s_t \quad c_u \quad s_r \quad e^+ \quad c_a \end{cases}$	$\begin{matrix} 76 \\ 46 \end{matrix}$	1, 6 $\begin{cases} h \quad t_h \quad s_t \quad c_u \\ r_u \quad h \quad D \quad s_r \quad e^+ \quad c_a \end{cases}$	$\begin{matrix} 19 \\ 17 \end{matrix}$
3 $\begin{cases} r_u \quad h \\ D \quad t_h \quad s_t \quad c_u \quad s_r \quad e^+ \quad c_a \end{cases}$	$\begin{matrix} 6 \\ 6 \end{matrix}$	2, 6 $\begin{cases} t_h \quad s_t \quad c_u \\ r_u \quad h \quad s_r \quad e^+ \quad c_a \end{cases}$	$\begin{matrix} 15 \\ 3 \end{matrix}$
4 $\begin{cases} r_u \quad h \quad t_h \\ D \quad s_t \quad c_u \quad s_r \quad e^+ \quad c_a \end{cases}$	$\begin{matrix} 11 \\ 2 \end{matrix}$	3, 6 $\begin{cases} D \quad t_h \quad s_t \quad c_u \\ r_u \quad h \quad t_h \quad s_t \quad s_r \quad e^+ \quad c_a \end{cases}$	$\begin{matrix} 1 \\ 11 \end{matrix}$
5 $\begin{cases} r_u \quad h \quad t_h \quad s_t \\ D \quad c_u \quad s_r \quad e^+ \quad c_a \end{cases}$	$\begin{matrix} 44 \\ 28 \end{matrix}$	5, 6 $\begin{cases} D \quad c_u \\ r_u \quad D \quad c_u \quad s_r \quad e^+ \quad c_a \end{cases}$	$\begin{matrix} 5 \\ 9 \end{matrix}$
6 $\begin{cases} r_u \quad h \quad t_h \quad s_t \quad c_u \\ D \quad s_r \quad e^+ \quad c_a \end{cases}$	$\begin{matrix} 46 \\ 87 \end{matrix}$	1, 5 $\begin{cases} h \quad t_h \quad s_t \\ h \quad D \end{cases}$	$\begin{matrix} 6 \\ 2 \end{matrix}$
7 $\begin{cases} r_u \quad h \quad t_h \quad s_t \quad c_u \quad s_r \\ D \quad e^+ \quad c_a \end{cases}$	$\begin{matrix} 41 \\ 57 \end{matrix}$	2, 5 $\begin{cases} t_h \quad s_t \\ h \quad D \end{cases}$	$\begin{matrix} 2 \\ 6 \end{matrix}$
8 $\begin{cases} r_u \quad h \quad t_h \quad s_t \quad c_u \quad s_r \quad e^+ \\ D \quad c_a \end{cases}$	$\begin{matrix} 77 \\ 218 \end{matrix}$	1, 2 $\begin{cases} h \quad D \\ r_u \quad t_h \quad s_t \quad c_u \quad s_r \quad e^+ \quad c_a \end{cases}$	$\begin{matrix} 6 \\ 1 \end{matrix}$
Total single crossovers	945	Total double crossovers	528
1, 8 $\begin{cases} r_u \quad D \quad c_a \\ h \quad t_h \quad s_t \quad c_u \quad s_r \quad e^+ \end{cases}$	$\begin{matrix} 110 \\ 23 \end{matrix}$	1, 6, 8 $\begin{cases} r_u \quad D \quad s_r \quad e^+ \\ h \quad t_h \quad s_t \quad c_u \quad c_a \end{cases}$	$\begin{matrix} 7 \\ 1 \end{matrix}$
2, 8 $\begin{cases} r_u \quad h \quad D \quad c_a \\ t_h \quad s_t \quad c_u \quad s_r \quad e^+ \end{cases}$	$\begin{matrix} 53 \\ 23 \end{matrix}$	2, 6, 8 $\begin{cases} r_u \quad h \quad D \quad s_r \quad e^+ \\ t_h \quad s_t \quad c_u \quad c_a \end{cases}$	$\begin{matrix} 4 \\ 4 \end{matrix}$
3, 8 $\begin{cases} r_u \quad h \quad c_a \\ D \quad t_h \quad s_t \quad c_u \quad s_r \quad e^+ \end{cases}$	$\begin{matrix} 3 \\ 1 \end{matrix}$	1, 7, 8 $\begin{cases} h \quad t_h \quad s_t \quad c_u \quad s_r \quad c_a \\ t_h \quad s_t \quad c_u \quad s_r \quad c_a \end{cases}$	$\begin{matrix} 1 \\ 2 \end{matrix}$
4, 8 $\begin{cases} D \quad s_t \quad c_u \quad s_r \quad e^+ \\ r_u \quad h \quad t_h \quad s_t \quad c_a \end{cases}$	$\begin{matrix} 2 \\ 27 \end{matrix}$	1, 5, 8 $\begin{cases} r_u \quad D \quad c_u \quad s_r \quad e^+ \\ h \quad t_h \quad s_t \quad c_a \end{cases}$	$\begin{matrix} 3 \\ 3 \end{matrix}$
5, 8 $\begin{cases} D \quad c_u \quad s_r \quad e^+ \\ r_u \quad h \quad t_h \quad s_t \quad c_u \quad c_a \end{cases}$	$\begin{matrix} 7 \\ 13 \end{matrix}$	2, 5, 8 $\begin{cases} t_h \quad s_t \quad c_a \\ r_u \quad h \quad t_h \quad s_t \quad s_r \quad e^+ \end{cases}$	$\begin{matrix} 2 \\ 1 \end{matrix}$
6, 8 $\begin{cases} D \quad s_r \quad e^+ \\ D \quad e^+ \end{cases}$	$\begin{matrix} 26 \\ 13 \end{matrix}$	5, 6, 8 $\begin{cases} r_u \quad h \quad t_h \quad s_t \quad s_r \quad e^+ \\ D \quad c_u \quad s_r \quad c_a \end{cases}$	$\begin{matrix} 1 \\ 1 \end{matrix}$
7, 8 $\begin{cases} r_u \quad h \quad t_h \quad s_t \quad c_u \quad s_r \quad c_a \\ r_u \quad D \quad e^+ \quad c_a \end{cases}$	$\begin{matrix} 3 \\ 28 \end{matrix}$	5, 7, 8 $\begin{cases} D \quad c_u \quad s_r \quad c_a \\ h \quad D \quad c_a \end{cases}$	$\begin{matrix} 1 \\ 1 \end{matrix}$
1, 7 $\begin{cases} h \quad t_h \quad s_t \quad c_u \quad s_r \\ r_u \quad h \quad D \quad e^+ \quad c_a \end{cases}$	$\begin{matrix} 18 \\ 12 \end{matrix}$	1, 2, 8 $\begin{cases} h \quad D \quad c_a \\ r_u \quad h \quad D \quad c_u \quad s_r \end{cases}$	$\begin{matrix} 2 \\ 2 \end{matrix}$
2, 7 $\begin{cases} t_h \quad s_t \quad c_u \quad s_r \\ r_u \quad h \quad e^+ \quad c_a \end{cases}$	$\begin{matrix} 9 \\ 2 \end{matrix}$	1, 5, 6 $\begin{cases} h \quad t_h \quad s_t \quad s_r \quad e^+ \quad c_a \\ t_h \quad s_t \quad s_r \quad e^+ \quad c_a \end{cases}$	$\begin{matrix} 2 \\ 1 \end{matrix}$
3, 7 $\begin{cases} D \quad t_h \quad s_t \quad c_u \quad s_r \\ D \quad e^+ \quad c_a \end{cases}$	$\begin{matrix} 1 \\ 1 \end{matrix}$	2, 5, 6 $\begin{cases} t_h \quad s_t \quad s_r \quad e^+ \quad c_a \\ h \quad D \quad s_r \quad e^+ \quad c_a \end{cases}$	$\begin{matrix} 1 \\ 1 \end{matrix}$
Total triple crossovers	36	Total quadruple crossovers	1
Grand total	2029		

TABLE 17

$\frac{B_l}{+} \frac{D}{+} \frac{+}{e_y} \sigma \times \frac{+}{+} \frac{+}{+} \frac{e_y}{e_y}$. Summary of counts in the "normal" cultures.

	WILD TYPE	B_l	D	e_y	$B_l D$	$D e_y$	$B_l e_y$	$B_l D e_y$
Females	1152	1145	1163	1052	1191	986	1023	1016
Males	1044	1038	1083	1015	1133	911	1117	975
Total	2196	2183	2246	2067	2324	1897	2140	1991

Grand total 17044

SEX LINKAGE IN MAN¹

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INTRODUCTION

The fact that chromosomes play an extraordinarily important part in determining development is now established. Not only chromosomes, as a whole, thus function, but also their constituent genes play a specific rôle. And each such gene plays a rôle that, on the one hand, is general in the development of the organism and, on the other hand, specific for a particular morphological or chemical quality of the organism.

Each gene, we must believe, is, ordinarily, located in a particular chromosome. One of these chromosomes is found in most of the higher organisms to be to a large degree determinative of sex. It is, accordingly, called the

¹ Based in part on field work done by Mr. C. V. GREEN under a grant made by the Research Committee, AMERICAN MEDICAL ASSOCIATION, Dr. LUDVIG HECKTOEN, Chairman.

sex-chromosome. A number of genes are located in the sex-chromosome and they are linked with sex itself in an interesting and curious way. In humans the female somatic cells have two sex-chromosomes (XX), one from each parental germ cell, while the male has only one X-chromosome and this is derived from the egg. It is associated with the Y-chromosome which carries few active genes.

A consequence of these relations is that sperm cells that carry an affected X-chromosome become progenitors of daughters half of whose eggs, in turn, carry the affected X-chromosome. If such eggs produce males, the males will be affected; if females, their defective X-chromosome will be balanced by a normal X-chromosome from the sperm and the females will either not show somatically or phaenotypically the defect that they carry, or will show it in a low grade of expression (see figure 1).

Now all of the sex-linked traits are linked, not with sex alone but also with each other. Accordingly we expect to find sex-linked traits associated with each other in inheritance.

It was this consideration that led the Research Committee of the AMERICAN MEDICAL ASSOCIATION to start a study of some human families with haemophilia to learn if color-blindness, or any other sex-linked trait, was in them linked with the haemophilia. Before giving our results (which were not as conclusive as hoped for) it may be well to review our knowledge of the best studied sex-linked traits in man. These are: color-blindness, haemophilia, optic nerve atrophy; also, but less extensively studied, night blindness, some cases at least of muscular atrophy, hypoplasia of white brain substance (Pelizaeus-Merzbacher disease), and some strains of myopia and of multiple sclerosis. Still other sex-linked traits have been cited, but the evidence for them is still very limited.

It is proposed to consider the best known of these sex-linked traits.

COLOR-BLINDNESS

It has been known for over a century and a quarter that some persons are defective in the color sense. Three main types of color defect are known.

(a) Achromatic vision. The central region (fovea) is alone sensitive to light. The loss of function is probably due to defective development of the cones. This condition is not known to have any hereditary factor.

(b) Red-green color-blindness; dichromatic vision. This includes the red-blind and the green-blind. Violet-blind persons have been described, but the condition is very rare. The commonest sub-type is red-blindness, called protanopia or blindness to the first essential (red) constituent of the

spectrum. Persons thus affected see the red end as an extremely low, unintense yellow. The green band also appears yellow and bright. The other sub-type includes green-blindness, called deuteranopia, or blindness to the second essential (green) constituent of the spectrum. Persons thus affected see the red end as a yellow that is only slightly reduced in intensity from normal yellow, while the green band is a yellow that is much less bright than in the case of the red-blind.

(c) Includes anomalous trichromatic vision. Persons of this type see the red, yellow and green of the spectrum; but red and green mixed in the proportions that give yellow to the normal eye do not give a good yellow to them. Two sub-groups can be distinguished here also.

Persons of one sub-group say that the mixed color is green; to make yellow more red of the spectrum must be added—this makes a yellow that is rather dull. Such persons are called “green-see-ers” or protanomals.

Persons of the other sub-group say the mixture of green and red that ordinarily gives yellow appears to them red. More green has to be added; then they see a bright yellow. They are called “red-see-ers” or deuteranomals.

The genetic problem is the relationship of these five conditions, normals, protanomals and protanops, on the one hand, and deuteranomals and deuteranops, on the other.

Before entering more deeply into the problem it is necessary to consider the statistics of color-blindness in the general population. It is commonly said that, in Europe generally, there are 4 percent of men and 0.4 percent of women affected; that is, one tenth as many women as men.

DANFORTH (1924) has argued that there is something incompatible with these proportions and the probability that the proportions of color-blind in the population are not changing. It may be said, parenthetically, that since color-blindness has been measured in large numbers of children only during the present generation we really have no basis for the conclusion that the proportion is not changing.

On the hypothesis that red-green color-blindness is due to a mutation in a gene of the sex chromosome (the X-chromosome) then the following considerations will hold. Since there is only one X-chromosome in the male, there is the same proportion of affected X-chromosomes in the male population as there is of color-blind males, namely 4 percent. Also it may be said that, for the same reason, 4 percent of the X-bearing male sperm of the entire population are defective in the gene for color discrimination that is carried in the X-chromosome. The proportion of normal to defective color genes in the male X germ-plasm is, therefore, as 96:4.

Since male zygotes derive their X-chromosomes exclusively from the egg, then in the whole population the proportion of eggs containing a defective X-chromosome is 4 percent; 96 percent have an unaffected X-chromosome.

In female zygotes there are 3 possible combinations (since each female zygote has 2 X-chromosomes). Since in all X-bearing sperm cells we have the ratio 96 percent X:4 percent X' and in all eggs 96 percent X:4 percent X' (where X' refers to the X-chromosome carrying the affected gene) then in all zygotes we shall have normals 0.96×0.96 or 0.9216. Of conductors 2 times 0.96×0.04 or 0.0768. Of color blind 0.04×0.04 or 0.0016.

The above computation gives us 0.16 percent color-blind females in the population instead of the 0.4 percent alleged to occur. We see, thus, that a population consisting of 4 percent color-blind men and 0.4 percent color-blind women will produce only 0.16 percent color-blind women in the next generation, while the proportion of affected men remains at 4 percent. Thus the proportion of color-blind women to color-blind men will tend rapidly to diminish in successive generations; but if it does, the proportion of color-blind men will also diminish.

SCHIÖTZ(1922) by means of refined methods of testing for color-blindness, reaches a conclusion that 10 percent of males and 1 percent of females are color-blind. Such a pair of ratios can maintain themselves approximately in successive generations. Doubt has been cast, however, upon the reliability of SCHIÖTZ's determinations.

WAALER (1927) has examined 9000 children in the schools of Oslo using the Ishihara test as a routine test and examining by means of the Nagel anomaloscope all whose reactions to the Ishihara tests were not normal. He also examined the entire family, as far as possible, of all of the boys and the girls who had defective color discrimination.

All males proved themselves to be either normal or to belong to one of the four aberrant types: protanomal, protanops, deuteranomal, deuteranops; and, in transmission, there proceeds from each male a chromosome of a type corresponding to the condition that the male shows somatically.

The females, however, do not show somatically or phaenotypically the whole content of abnormality in the X-chromosome since they have two such chromosomes and these may be either similar or unlike. The composition of the X-chromosomes of the mother is demonstrated by the color discrimination of her sons. Also the color discrimination of her father and brothers will help with the analysis, since she receives one of her X-chromosomes from her father and one from her mother, whose constitution is in part indicated by the condition of her brother. As a result of this colossal investigation of WAALER's it appears that the protanomal condition is

dominant over the protanops; the deuteranomal is dominant over the deuteranops; while the normal condition dominates over all. The normal, the protanomal and the protanops constitute three members in an allelomorph series. The gene for each is located in the same point of the chromosome. Similarly, normal, deuteranomal, and deuteranops constitute an allelomorph series located in the same gene. The author is inclined to conclude that the genes for all five conditions are identical but suffer varying amounts of kinds of damage.

WAALER's statistics indicate that 8 percent of the population of boys examined show some type of abnormality in color discrimination. One would expect 0.64 percent of the girls to show color-blindness. There are actually found $0.44 \text{ percent} \pm 0.07$. The deviation from expectation, while considerable, is not too improbable in view of the considerable size of the probable error. The statistical findings of WAALER, therefore, show a relation between abnormal males and females that can persist in successive generations.

Anatomical basis of red-green color-blindness

A thorough critical examination of the literature, with a study of some new families, has been recently made by BELL (1926). She points out that it is uncertain upon just what modifications of the normal color discriminating apparatus color-blindness depends. Indeed, the basis of normal color discrimination is not certainly known. There is some evidence that the cones of the retina are part of the color discriminating apparatus. Thus LARSEN (1921) found that in totally color-blind persons, whose eyes he sectioned, the cones of the fovea "were short and plump where they are normally found to be long and slender." Also they had either very short outer members or these were wholly absent. There is some evidence that a central-cerebral defect is also involved, since "disturbances of cerebral origin from pathological processes may cause total color-blindness without any affection of the retinal structures." (BELL 1926, p. 172).

Inheritance of red-green color-blindness

Color-blindness is commonly considered to be due to a recessive gene mutation located in the sex chromosome.

If the father is color-blind, his sole X-chromosome carries the defective gene. If the mother is color-blind, both of the two X-chromosomes, by hypothesis, carry the defective gene. But a mother may carry the defective gene in one of her X-chromosomes, while the other is unaffected. Such a mother, called a conductor, will be phaenotypically normal.

While all the eggs carry an X-chromosome, only half of the sperm cells do (the other half carrying the Y-chromosome which plays a small rôle in inheritance). The sons get their single X-chromosome from the egg only; so if the mother is without gametic defect none of the sons will be color-blind even if the father is. The daughters get an X-chromosome each from the egg and sperm. If the X-chromosomes of the egg are all without the gene defect, then the daughters can distinguish colors even if the sperm cell brings in a gene-defect in its X-chromosome. In the latter case, the heterozygous daughter can pass on the defective X-chromosome to half of her offspring. In the case of the sons half will be color-blind; in the case of the daughters half will be conductors—forming some eggs containing the defective X-chromosome.

Do the heterozygous females always have normal color discrimination? BELL (1896, p. 195) cites one sibship containing three color-blind females, the father of whom has been definitely tested by NETTLESHIP (1912) and pronounced to have normal color vision; and in several unquestionable cases color-blind women have been demonstrated to have some sons with normal color vision. In BELL's (1926, p. 233) pedigree (chart 418, II 4), a woman, aged 54, was thoroughly tested by wools, Nagel's cards and the Stilling test and found to be definitely color-blind; she required the help of others in choosing her dresses. She had a number of daughters and 7 sons. Three of these sons are clearly color-blind. Of the 4 others 3 were tested for color-blindness. The first was tested by Sir J. H. PARSONS, who found him normal with Holmgren's wools, Stilling test and colored lights. The other 2 boys were given an equally rigid test and showed fine color discrimination. Of the 4 daughters tested all were found normal. The condition of color vision of the parents of the affected mother is not definitely known. On the chromosome theory of heredity, it seems most probable that (unless the father was color-blind) the mother was heterozygous for color discrimination, and that the normal gene is imperfectly dominant. Thus we conclude that heterozygous females sometimes lack perfect color discrimination.

In figure 440 of BELL's work (1926, p. 238) the "mother" (VI 13) is color-blind and has a color-blind father and a carrier mother; and at least 2 of her 3 sons are color-blind. Except that *all* sons should be color-blind, this accords with expectation, since in the "mother" both X-chromosomes are probably affected.

A summary of cases given by BELL is instructive. When both parents are color-blind all (4) sons and (5) daughters are color-blind. When the father is affected and the mother's stock is so likewise, 49 percent of sons

and 37 percent of daughters are affected. When both parents are normal and the mother's stock is affected 61 percent of the sons and 2 percent of the daughters are affected. Even when the mother is affected and the father normal only 3 percent of the daughters are affected. There is no doubt that color-blind fathers have carrier daughters who, in turn, have color-blind sons. That is, the defective chromosome passing through the (heterozygous) daughter is transmitted to half of her sons, who thus lack capacity for color discrimination.

HAEMOPHILIA

Normally the blood flows in well circumscribed, walled spaces in the body—the blood vessels; if the walls of the vessels are cut blood has the property of making a temporary wall, or plug, by clotting.

The irregularities in the physical behavior of blood are due (1) to weaknesses, or abnormalities, of the vessel walls, permitting extravasation into the tissues; (2) to abnormalities of the clotting process or (3) to a combination of the two preceding.

The first and third classes of cases are recognized by the symptom of purplish spots arising in the skin in consequence of such extravasation of blood. The commonest types are known as purpuras, or purple spot diseases.

Of the purpuras, the first type is characterized by the tendency to form purple specks which flow together to form larger patches—usually on the legs and arms and often symmetrically. In extreme cases violent intestinal and gastric symptoms—pain and vomiting—occur. The patient usually recovers in a few days. The symptoms are accounted for on the ground that bodily toxins cause paralysis of capillaries, or larger vessels; so that tone and resistance to pressure are lost. Probably the loss of tone is due to a preceding intoxication of the spinal innervation of these finer blood-vessels. There is no evidence of an hereditary tendency.

In the other type of purpura and in the so-called haemophilia, there is an abnormality in the blood which interferes with normal clotting, so that the clotting or coagulation time is greatly delayed or clotting wholly inhibited. (*For the determination of coagulation-time.* There are several methods in use, such as those of VIERORDT, W. SCHULTZ, BÜRKE, MORAWITZ and BIERICH, WÖHLISCH, SAHLI-TONIO, KOLLMANN and others. The salt-plasm method of WOOLRIDGE and NOLF is highly spoken of by BAAR and STRANSKY (1928). It is applied as follows: The blood flowing from the vein of the arm is received into an equal quantity of 10 percent NaCl solution (2 marks on the test tube) and smartly centrifuged. There results

a salt-plasma that does not spontaneously coagulate. To test coagulation, 1 cc of this salt-plasma is diluted with 4 cc of distilled water in a test tube that has been well cleaned with alcohol and ether and dried. One drop of egg yolk is added. Every 5 minutes one observes whether or not the plasma is coagulated, by gentle inclination of the test tube. It is desirable always to make 2 parallel determinations and 2 controls with normal blood. Normal salt-plasma coagulates within half an hour; haemophilic blood often remains uncoagulated or shows after many hours to some days an often incomplete coagulation.)

Clotting is due to the formation of threads of fibrin in the blood plasma which entangle the corpuscles and form a plug. This is an invaluable property in preventing bleeding to death at wounds; but it is a dangerous property if it occurs inside of blood vessels, since the plug might well enter into and close up essential vessels, causing perhaps instant death. Fibrin is formed, however, only in the air; and by an interaction between a substance in the blood plasma and a substance in some of the cellular elements of the blood, particularly the blood platelets, which rapidly disintegrate in the presence of air. The substance in the plasma is called fibrinogen and the enzyme from the cells is called thrombin. It is the interaction of the two that produces fibrin.

Just as fibrin is formed only out of the blood plasma in the air, so thrombin is formed in the cells, but only in the presence of calcium. The calcium is necessary to the activation of the thrombin. Blood collected in a vessel containing a weak solution (a little over 0.1 percent) sodium oxalate will not clot; but if a saturated solution of sodium chloride be added it clots at once. It is believed that the calcium is necessary to the formation of the thrombin out of something in the cell—and this something HOWELL calls prothrombin or “thrombinogen.” It is also called, by BORDET, (1920) *zytozym*.

It is HOWELL'S (1913) hypothesis that the prothrombin of the blood is ordinarily prevented from forming thrombin by a substance that he calls anti-thrombin. But in the air, cellular elements, or the plates in the blood, neutralize the action of the anti-thrombin and, in the presence of calcium, enable the prothrombin to form thrombin.

In the second type of purpura, sometimes called purpura haemorrhagica, or thrombopenic purpura, there occur spontaneous bleedings in skin (forming purple patches); at mucous membranes—nose, gums, etc.; and internal organs—such as kidney and retina. Usually there is no bleeding at joints as in haemophilia. An histological study of such blood indicates that the number of blood plates is much reduced—so that

thrombin can not be formed in normal amount. It is on this account that this disease is sometimes called thrombopenic purpura.

An hereditary history for this disease has been given in a number of cases, which indicate perhaps a dominant heredity. Possibly in one or more of the genes of an autosome there is a positive something that prevents the formation of blood plates in normal amount and thrombin-forming function.

In *haemophilia* the symptoms are:

(1) After a cut, a persistent hemorrhage occurs that is scarcely controllable by artificial means. From the smallest wound the blood may trickle for days and weeks.

(2) Spontaneous hemorrhages appear, especially, persistent nose bleed; also striking effusions into the joints, which become enlarged and blackened—"blood joints," or "bleeder joints."

Probably only males are affected and most affected males (60 percent) die before the age of 8 years. In the 11 percent that attain the age of 22 years, the symptoms are ameliorated and after about 40 they are never severe.

The degree of symptoms is variable and in respect to symptoms biotypes appear in various families.

Cause of haemophilia

BAAR and STRANSKY (1928) after examining the results of all experimental work on the subject conclude:

Neither the fibrogen content of the haemophilic blood, nor the thrombin content of the haemophilic serum is diminished. No increase of coagulation-inhibiting factors is found.

The disturbance of coagulation in haemophilia is thus to be sought in the first phase of thrombin formation.

Although there are divergent experimental results, BAAR postulates as most probable, an insufficiency of a cell enzyme (prothrombin of HOWELL or zytozym of BORDET) and its retarded relinquishment by the blood plates.

From this point of view there is a gene in the X-chromosome that determines the production of a normal amount (and kind) of zytozym (prothrombin) in the cells and especially cellular elements of the blood. In haemophilia this gene is modified—and in different degrees—resulting in biotypes of haemophilia of varying degrees of defect.

Two rules of inheritance have been suggested.

NASSE's rule: Only males are affected; the disease is transmitted through unaffected daughters to their sons.

LOSSEN's rule. No affected male has direct male descendants who are affected. The transmission is only from female to female or female to male; never from a male to his daughters.

It should be easy, one would think, to decide between the rules of NASSE and LOSSEN by examination of the pedigree charts that have been published. But such examination leads to much disappointment. Of the 235 pedigree charts published in the work of BULLOCK and FILDES (1911) the only ones that show grandchildren of a certainly affected male are: Nos. 373 (TENNA family), 382, 387, 389, 402, 408, 424, 426, 431, 436, 445, 462, 465, 468, 474, (478), 490, 493 bis, 512, 528, 535, 540, 541, 548, 562, 570, 581, 584. But No. (478) is regarded as a valueless pedigree by BULLOCK and FILDES. In the 27 significant pedigrees there are 31 affected males who have grandchildren. Together these 31 males have 47 daughters that have children. Together these daughters have 128 sons, and of these $78 \pm$ are affected. Thus there are $78 \pm$ haemophilic grandsons of affected males descended in the female line from affected grandfathers. Certainly this result is opposed to LOSSEN's rule: "Nur Männer sind Bluter, vererben aber, wenn sie Frauen aus gesunden Familie heiraten, die Bluteranlage nicht." Of the 31 affected male progenitors of grandchildren there were only 3 who did not have affected grandsons. It is quite impossible to believe that of these 31 men 28 (or 90 percent) married wives belonging to haemophilic families. On the other hand there are some statistical irregularities in the pedigrees. Expectation is that one-half of the sons of "conductor" mothers will be haemophilic. Of 128 sons recorded, $78 \pm$ (some uncertainties) are regarded as bleeders. This is 14 more than expectation. Also, of these "conductor" mothers the daughters who have any haemophilic sons should have them in the proportion of 50 percent of all sons. Actually of 45 recorded 34 are given as bleeders. These deviations from expectation are too great to be due merely to insufficient numbers. It seems probable that a larger proportion of haemophilic sons than of normal sons is recorded. The recorder of the pedigrees has his attention focussed on bleeders in the family, he records all such; but he is less careful to inquire into and record non-bleeders.

It seems the most probable conclusion that haemophilia follows the usual course of sex-linked inheritance, which is stated in NASSE's rule.

An important inquiry is: Where are the haemophilic females? Such are to be expected from certain matings, just as color-blind females arise from certain matings. Although bleeding females have been described, careful

analysis usually shows that they are either women affected with purpura or else they are very incomplete cases of true bleeding appearing in "con-

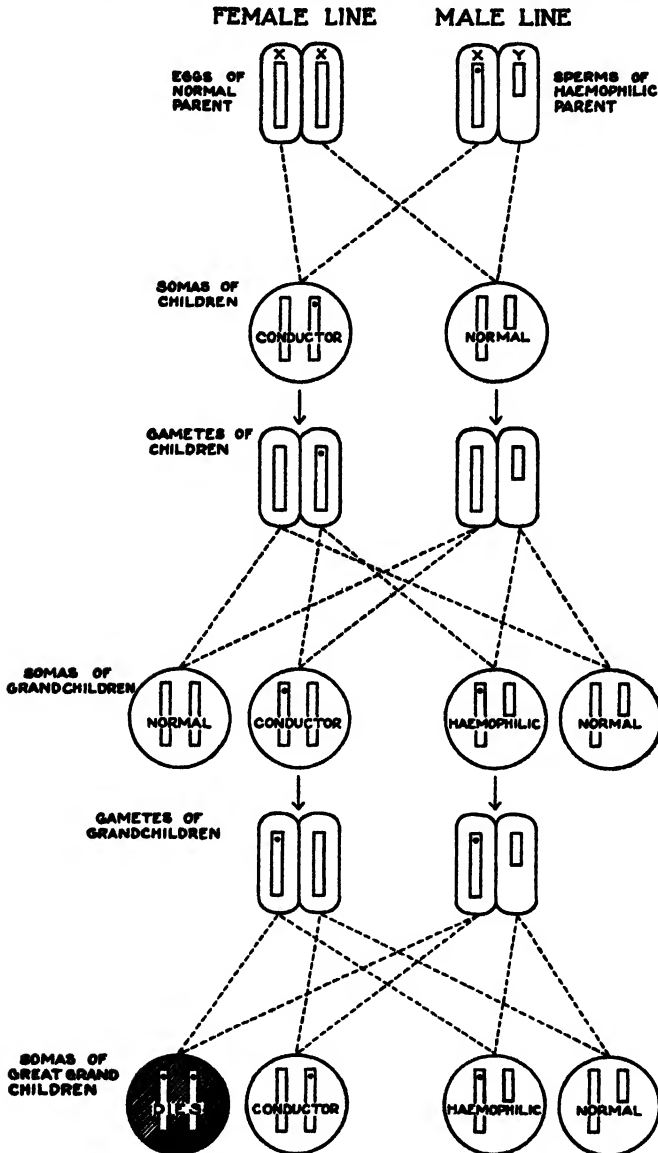


FIGURE 1.—Scheme of inheritance along male and female lines of the gene for haemophilia. Drawing by Miss M. B. CHAMBERS.

ductors" (that is heterozygous females). The most reasonable explanation of the absence of typical haemophilic symptoms in the female is that the

zygote that receives 2 affected X-chromosomes dies early, usually before birth (figure 1).

HEREDITARY OPTIC NERVE ATROPHY (LEBER'S DISEASE)

This disease commonly begins at adolescence with a loss of clear vision, due to the development of an area of partial, or total, blindness (scotoma) in the axis of vision. Just because this area is that of greatest color discrimination, color vision is greatly reduced. On ophthalmoscopic examination the disk of the optic nerve is found to be grayish or whitish instead of pink. In later stages the margins of the disk become ill defined; also the blood vessels of the retina become narrowed and more tortuous.

Hereditary optic atrophy belongs to a genus of diseases that is characterized by degeneration of the optic nerve. In the genus are also (1) intoxication nerve atrophy, also called "*neuritis axialis chronica sive actua*," and (2) diabetic nerve atrophy.

Intoxication nerve atrophy appears subsequent to excessive use of alcohol or tobacco. Wood alcohol blindness belongs in this category. After abstinence vision is improved. In the hereditary form no improvement follows abstinence (though abuse of narcotics often precedes its onset). Also the narcotic type is often one-sided, while the hereditary type is bilateral—though vision may be affected in one eye earlier than the other. Moreover in the narcotic form the sexes are nearly equally affected; while in the hereditary type females are not usually affected. Also in the narcotic type onset is ordinarily slower and occurs later in life, and vision is less often completely impaired.

The diabetic species of optic nerve atrophy is associated with a high percentage of blood sugar and affects the sexes equally.

The specific diagnosis of hereditary optic atrophy is based on the inability to control it by abstinence from narcotics, or on its non-association with high blood sugar, on a relatively early and sudden onset (usually under 28 or even 25 years) and on a prevailing tendency to affect several males in the same family and, much more rarely, females. Thus in a number of affected fraternities, of 537 sons who grew up 390, or 72.6 percent, were known to be affected; whereas in 428 matured daughters of the same fraternities only 32 daughters, or 7.5 percent, were affected (table 1). Of the affected, 92 percent were thus males and 8 percent females. Again WILBRAND and SAENGER (1913) found in 308 affected cases 272 males and 36 females, or 88 and 12 percent respectively. The population of persons affected with optic atrophy is divided into males and females roughly in the proportion of 9 to 1.

The diminution of vision usually proceeds farther in the case of optic atrophy than in the case of narcotic amblyopia, so that fingers can not be counted at a greater distance than 3 meters. In narcotic amblyopia vision is usually reduced one-half. But the best diagnostic criteria of Leber's disease is its hereditary quality.

Incidentally it may be mentioned that biotypes of hereditary optic atrophy seem to occur. Some biotypes are characterized by early onset (at 8 or 10 years); others by prevailing late onset (35 to 50 years). Some biotypes are characterized by cessation of degeneration at an early stage—with persistence of slight amblyopia; others by practically complete blindness. Apparently there are biotypes in which the heterozygous female tends to show optic atrophy. The existence of the biotypes reminds one of allelomorphic series, of which the more nearly normal representatives are dominant over the more defective.

The enormous predominance of males early led to the conclusion that Leber's disease is a sex-linked one.

In typical sex linkage the percentage of incidence of the female sex is equal to the square of the incidence of the male sex. The reason for this is that while X' indicates the incidence of the single affected sex chromosome that causes the sex-linked trait in $(1X)$ males, $X'X'$ indicates the percentage incidence of two affected X' -chromosomes necessary to the production of $2X$ -females who show the sex-linked trait. Nobody has computed precisely the proportion of mature males in the native male population who have optic atrophy. Assume it as .005, or 5 per thousand of the population, then the proportion of females would be 25 per million. In other words the affected females would be $1/200$ th as common as affected males. The tables that I have collected contain about 75 affected females and 481 affected males. However, not all of these females have been seen. FLEISCHER and JOSENHAUS found in the literature only 6 cases of females that had been directly investigated. But KAWAKAMI (1926, p. 576) has the opinion that in Japan the incidence of females is much higher than in the European cases considered by FLEISCHER and JOSENHAUS. In any case, in the selected families affected females are about $1/10$ th instead of $1/200$ th as numerous as males. This result may be due to the expression of the trait in many heterozygous females.

Let us now consider the incidence of affected males and females following given matings in which affected X -chromosomes are probably involved.

The question has been frequently raised whether optic nerve atrophy follows the law of NASSE, like color-blindness; or the law of LOSSÉN,

according to which an affected man has no affected descendants in first, second, or later generations (unless intermarriage occurs with females of affected stock).

Tables 2a to 2e contain data drawn from the pedigrees given for families whose defect is most probably true Leber's disease. In selection of families use was primarily made of the critical examination of DREXEL (1922). To DREXEL's accepted cases are added certain earlier ones not considered by him and a number of families that have been published since his work. KAWAKAMI (1926) has published pedigree charts for a lot of cases that had appeared in the Japanese literature and had been overlooked. These I have not included. Most of them are fragmentary and, in general, the incidence of affected females is so high that they constitute a class quite different from the European and North American cases. I have, however, included KAWAKAMI's own case. One wonders whether the Japanese physicians have a somewhat different criterion of Leber's disease from the European or whether the factor, or factors, that make for hereditary optic atrophy lie in different chromosomes in the peoples of the two continents.

Considering this tabulation it appears that in pedigrees where 50 percent of affected males are expected (the mother being a conductor) 63 percent and 66 percent are found in matings I and IV respectively. The unexpectedly large proportion of affected males may be due to a selection of data: namely, of just the affected brothers, the others being unreported. In the type of mating (II) where the mother is affected and therefore 100 percent affected males are expected—73 percent are found. This result might be interpreted to indicate that some of the affected mothers are only heterozygous.

In matings (I) where the father is normal and the mother a conductor, no affected daughters are expected, but 6 percent are found, clearly indicating that some heterozygous daughters are affected, even though atypically. Where the father is normal and the mother is affected (mating II) the proportion of affected daughters rises to 17 percent. In mating III (affected father, genotypically normal mother) 2.8 percent of the daughters are affected. These results are irregular. They suggest that either there are biotypes in which the heterozygous female is affected; or else a special accessory factor for blindness exists in some strains.

In mating III, where the father is alone affected, there are very few sons affected. In the 2 matings that yield affected sons the mothers are really unknown. Though they were almost certainly not affected they may readily have been conductors. In GINSBERG's family an affected man had

3 sons by one woman and 1 by another, all affected. We know nothing about these women though they were probably not affected. For all we know to the contrary they may have been sisters and both conductors. In the other families, comprising together 81 grown sons, none are affected. In view of our ignorance of the mothers the results of mating III are not opposed to NASSE's law.

Again, KAWAKAMI (1926, pp. 592-594) has tabulated the best known families with optic nerve atrophy, with special reference to the vision of the mother's father of an affected person. There are 56 such mother's fathers described and 47 not described. Only 4 are known to have been blind. KAWAKAMI contrasts this condition of not more than 7 percent with the 40 percent occurrence of known color-blind mother's fathers in color-blind families. This difference is the more striking since the proportion of known to actually atrophically blind is doubtless greater than the proportion of known to actually color-blind. The 4 cases of maternal grandfathers affected with optic atrophy may well be due to inheritance from the mother's mother as a conductor. The fact of much higher incidence of affected maternal grandfathers in color-blind families than in families with optic nerve atrophy is strong evidence that inheritance does not follow the same law in both cases.

MACKLIN (1927), despite this evidence, or perhaps without fully considering it, is inclined to doubt the validity of LOSSEN's law in opticus atrophy. She points out that usually too few generations are known or there are no male descendants of daughters of the affected man. This criticism is all too true. Still there is a remarkable absence of daughters of men with opticus atrophy who are certainly conductors. Referring to mating III, we find that in the family of USHER (1927) of 3 daughter's children of the blind grandfather not one was affected, although apparently of the age of incidence. Again in the family of HIRSCH (1923) the 3 sons of 2 daughters of an affected man (II 1) are unaffected; and again the married daughter of blind III 8 had 3 grown sons of whom none was affected. Also, of 5 other married daughters none of the 3 sons was affected.

Out of the hundreds of grandchildren of men with opticus atrophy the only sure cases of daughters' sons with opticus atrophy that I have been able to find in the literature (not explicable by the female lines) are the following:

In GOULD's (1893) case, in the first generation there is a man with opticus atrophy one of whose daughters (II 4) had 4 sons who survived to maturity and one of these (III 9, who died at 40) had opticus atrophy (figure 2).

In HANCOCK's (1908) case a man (I 1) who had optic atrophy had a normal daughter (II 1) whose 3 sons (III 1, 5, 8) all had optic atrophy. She had a daughter also (III 3) who had 4 sons of whom 1 had optic atrophy. A sister (III 7) of the last mentioned daughter's daughter had 2 affected sons. II 1 had another daughter, III 2, and her daughter (IV 3) had an affected son V 2 (figure 3).

These two families offer remarkable exceptions that only enforce the general rule that in typical Leber's disease affected males have no affected descendants. On the chromosome theory of inheritance, as indeed applied by KAWAKAMI (1926, p. 590), the union of a Y-chromosome with the

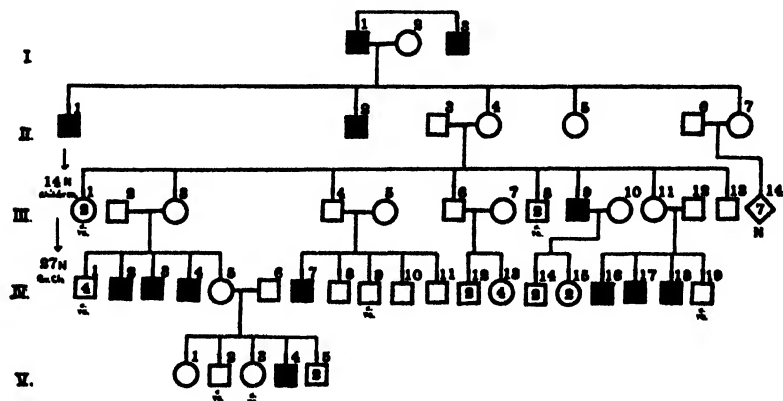


FIGURE 2.—Pedigree of optic atrophy, by GOULD, 1893. II 1, age at onset unknown; became blind from same type of disease which has since appeared in the later generations. His father was blind and deaf somewhat late in life. I 3 affected at 28 years, like his brother. II 1 affected, died at 86. II 2 affected at 28. II 4 eyes weak and watering; but not blind, died at 62 years. II 5, horned in eye by a cow; blind before 40. II 7 became blind late in life, cause unknown (probably cataract); no affected children. The later generations follow the rule of sex-linked inheritance; except that IV 7 offers difficulties since his sex chromosome was derived from his mother who, apparently, does not belong to the family with optic atrophy.

affected X-chromosome from a female conductor permits, indeed, the optic nerve atrophy to develop in the male. But somewhere in the development of the zygote or of its sex cells, or during maturation the affected X-chromosome becomes either modified or lost. Or it may be that the sperm cells that carry the affected X-chromosome do not function. At any rate, in the vast majority of cases the affected X-chromosome that has been associated with the Y-chromosome does not function again. Of course, if daughters of affected males produce no conductor-daughters; or such daughters produce few, or no, sons the same result would follow. In the families of GOULD and

of HANCOCK considered above the Y-chromosome does not experience its usual, but peculiar, fate.

Finally, there are a few families in which inheritance of optic nerve atrophy appears to follow a non-sex-linked and nearly, or perfectly, a dominant type of inheritance. The case of KNAPP (1904) is an example. Here an affected father has 2 affected sons out of 5; and 2 affected daughters out of 3. One of the affected daughters marries her normal cousin and they have 4 affected sons out of 5 and 1 unaffected daughter.

Again, WAARDENBURG (1924) tells of a family in which the grandfather, who was examined ophthalmoscopically, showed optic nerve atrophy. His daughter (also observed ophthalmoscopically) had a similar loss of

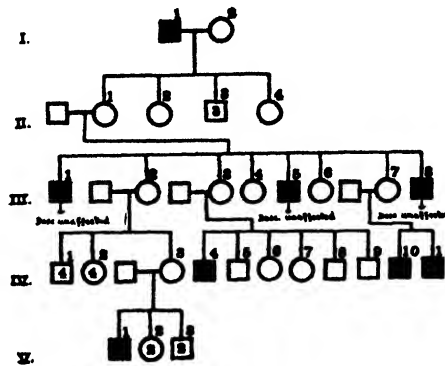


FIGURE 3.—Pedigree of optic atrophy, by HANCOCK, 1908. I 1 in third decade sight failed; later improved; lived to be 100 years old. III 1 vision failed in both eyes at between 20 and 30 years; never recovered, a heavy smoker. III 5 lost vision like III 1; after a year recovered sight; no abuse of alcohol or tobacco. III 8 attacked at between 20 and 30 years; not abstemious; no recovery. III 1 had 4 sons and 3 daughters, and he had 5 grandsons, none affected at the time of report.

vision. By a normal man she has 5 sons, 3 affected; and 5 daughters, 1 affected. This looks like a case of a dominant gene (instead of a recessive) located in an autosome (instead of X-chromosome). Genetically it is as different from ordinary Leber's disease as can well be.

KROPP (1927) described a family in which the father began to lose vision at 21 years, having a partial optic atrophy on both sides. His wife, so far as all tests go, has normal vision. They had 5 children. The first son and first daughter had normal vision. The second daughter had a neuritis back of the eyeball of unexplained etiology, and on both sides a central scotom. The third daughter had atrophic optic nerves, with relative central color scotom, on both sides. In this case the genetic condition is like that in WAARDENBURG's case cited in the last paragraph.

WAARDENBURG (1924, Stammbaum IV, V) gives two pedigrees where the marriage of unaffected first cousins has resulted in affected sons and daughters. These pedigrees suggest that the gene concerned is a recessive, autosomal one.

These aberrant cases are quite confusing. That a recessive, sex-linked gene might come to lie in an autosome is quite possible. A similar exchange of genes between non-homologous chromosomes has been described in *Datura* (BELLING and BLAKESLEE 1926), and elsewhere. That a gene responsible for continued functional vigor should in some cases by "weakness" (recessiveness) produce opticus atrophy and in other cases by "strength" (dominance) produce likewise the same type of opticus atrophy seems incredible. Possibly just a delicate median balance of functional activity of this gene is required for normal functioning of the optic nerve.

The conclusion of this analysis of the cases of Leber's disease is that it is generally a recessive, sex-linked trait with a usual failure to reappear in descendants of affected males. However, it is expressed occasionally in females who are "conductors" or heterozygous for the trait. Moreover, the trait of hereditary opticus atrophy is sometimes induced by a gene that is not located in the sex-chromosome.

OTHER SEX-LINKED TRAITS

Brief mention may be made of other sex-linked traits. No attempt is made here to analyze them or to extend the bibliography.

Hypoplasia of white brain substance, as worked out by MERZBACHER (1909), seems to be a case of sex-linkage, but so far confined to one family.

Night blindness, or hemerolopia of the sex-linked type, occurs in which males only are affected. This is not to assert that females can not be affected—the available pedigrees are very few in number. BELL (1922) lists only the great CUNIER-TRUC-NETTLESHIP pedigree, that of NETTLESHIP and MORTON and 29 minor ones. The large CUNIER-TRUC-NETTLESHIP pedigree of the NOUGARET family of VENDÉMIAN includes both males and females. There are 12 families in which the males only are affected. AMMANN's (1898) case is typical, with 14 affected males and no affected female. All affected males inherit through unaffected females. Incidentally, all affected males are said to be myopic, but this is based only on family tradition.

Myopia, or short sightedness, in some families, runs a sex-linked type of inheritance. Thus, in some of the night-blind families with affected males only there is also myopia in some (or all) of the affected males. Even without night blindness some families have been described, like

those of WORTH (1906) (see figure 4) and OSWALD (1911), in which males alone are myopic and inherit through unaffected females.

A sex-linked inheritance of megalocornia has been described by KAYSER (1914).

Pseudo-hypertrophic muscular paralysis of GOWERS (1879) is also sometimes sex-linked. In sex-linked families about 6 times as many males are affected as females. TSCHERNING (1921) has discussed inheritance in this disease.

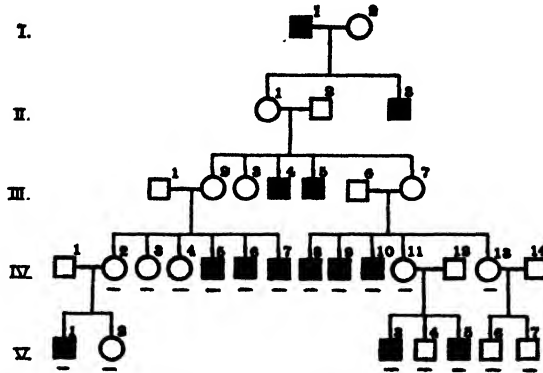


FIGURE 4.—Pedigree of family with hereditary sex-linked myopia (WORTH 1906). The black symbols indicate myopia. The underscored symbols represent persons examined. In addition some members of the III^d generation were seen. The other cases are classified on the basis of statements of relatives. The myopia was measured as 10 or 12 D, with some astigmatism.

Other traits for which sex-linkage, in certain strains, has been found are: coloboma, nystagmus, microphthalmia, ichthyosis, webbed toes, toothlessness, deficiency in sense of smell and wanderlust, or nomadism.

SOME NEW PEDIGREES OF HAEMOPHILIA

During the summer of 1927, under a grant from the Research Committee of the AMERICAN MEDICAL ASSOCIATION, Mr. C. V. GREEN undertook to trace families with haemophilia in order: (1) to check on them the method of inheritance and (2) to learn if in any of these families there was also color-blindness. The special purpose of the investigation was to find evidence of sex-linkage of these two characters.

Mr. GREEN took preliminary instruction in the determination of haemophilia from Professor W. H. HOWELL, to whom we desire to express our gratitude. For determination of color-blindness the Stilling Isochromatic plates were used. Mr. GREEN's itinerary took him to Dushore, Athens, Sayre and Wilkesbarre, Pennsylvania (July 12-15), to Findlay and Day-

ton, Ohio (July 18th and 19th), to Rushmore, Minn. (July 25), to Sibley, Iowa (July 26), to Frederickton and Des Moines, Iowa (July 28–August 1), to Williamsport, Pennsylvania (August 15), to Cleveland, Ohio (August 30, 31) and to Detroit (September 2). A considerable amount of time was spent on trips that yielded no result, owing to the removal of families.

R-family (figure 5)

The most extensive series of families included certain descendants of the well-known MOLYNEUX strain of Pennsylvania. A MOLYNEUX daughter married a distant cousin of the same MOLYNEUX stock and by him had 4 sons, half with haemophilia (II 3, 5) and 2 daughters (II 7, 8). A sister of the husband mentioned in the last sentence had 1 son (II 9), a bleeder.

II 3 lives in Wilkesbarre. At about 10 years of age he cut his finger badly and it was then first discovered that he was haemophilic. He has

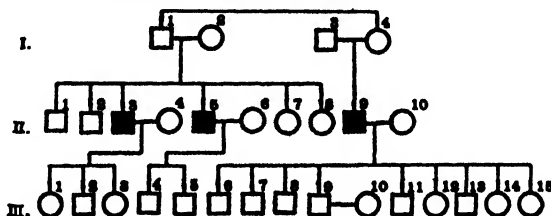


FIGURE 5.—R. family with haemophilia. The black squares indicate bleeders.

been twice given up to die by physicians, as a result of bleeding from extracted teeth. He has not been troubled lately as he takes very good care of himself (own statement). He was given the Stilling test and proved to have a very good color discrimination, much better than his wife's. One of their sons was given the Stilling test and did exceptionally well in it.

II 5 (brother of II 3) works at Athens, Pa. He has been in the hospital at Sayre on two or three occasions from hemorrhages, one of which was caused by a rather severe bruise on the shin. Blood collected until a large black lining was formed. When this was lanced a severe attack of bleeding followed. II 5 did only fairly well on the Stilling test; but quite as well as II 3's wife.

II 9 (cousin of the foregoing) is a well informed farmer, living near Dushore, Pennsylvania. He appreciates the method of inheritance of haemophilia. His worst attack of bleeding resulted from an infected finger, followed by "blood poisoning." On this occasion he nearly bled to death. His teeth are in bad condition but he hesitates to have them extracted,

fearing the probable consequences. He did fairly well on the Stilling test; better than his wife. Three of their 9 children, who were tested, did well.

The distribution of haemophilia in this family accords with the law of sex-linked inheritance of haemophilia; a typical sex-linkage confined to the male sex. None of the offspring of affected males are affected. Presumably the consorts of the affected males are genetically normal. There is no color-blindness in this family.

M-family

This is also a branch of the MOLYNEUX family, I 2 having been a MOLYNEUX before marriage. She had 7 sons. Of these 2 are known to have been haemophilic. II 8 was seen. He is affected by slight shaving cuts no more than is a normal person. His worst attack occurred about a year ago when he had his teeth extracted. At that time he was confined in a hospital at Sayre, Pennsylvania for a considerable period. It was feared that the hemorrhages would be fatal.

The color discrimination of II 8 is good, as good as that of his wife. Also the color discrimination of his daughter III 1 and son III 2 in each case is good—about the same as in the mother; the 9 year old sister made 2 more errors than her 7 year old brother.

P-family

The propositus, I 1, is a member of the MOLYNEUX family and lives in Detroit, Michigan. He is a pale, sickly man. His teeth are in very bad condition but he does not dare to have them extracted. He is troubled much with rheumatism. The slightest bruise causes swellings at the joints, especially at the knees and elbows, which are distended almost constantly. A slight cut on his hands causes a great amount of bleeding. When he was a child his mother had to watch at his bedside day and night for six weeks, as a result of having a milk tooth extracted. As a child he had an arm broken which could not be set right because of the excessive bleeding. According to his sister his whole life has been ruined on account of his defect. His case is the most severe of any seen among the MOLYNEUX. His color discrimination was not tested.

A-family

In this family, also one of the MOLYNEUX strain, the wife, I 2, is a sister of I 1 of the P. family. She married an unrelated man. They have 2 sons, ages 20 and 9 years respectively, and a daughter, aged 7. The second son, II 2, has haemophilia. He was first discovered to be a bleeder

when about 5 years of age. He had a deciduous tooth extracted and there was profuse bleeding for 9 or 10 days; a serum was successfully used to stop it. Bleeding at the mouth has several times occurred, especially around the teeth; and his pillow is sometimes badly stained with blood, or he has to leave school on account of bleeding at the mouth. Cuts on other parts of the body do not trouble him greatly. He bleeds from them hardly more than a normal person. He is not yet, at 9 years, troubled with swellings at the joints.

None of the family were color-blind. The largest number (about 13) of errors (Stilling test) was made by the 7 year old girl.

Gross family

The pedigree of this family is given in figure 6. It is the family to which most attention was paid. It is the only one in which color-blindness was certainly combined with haemophilia.

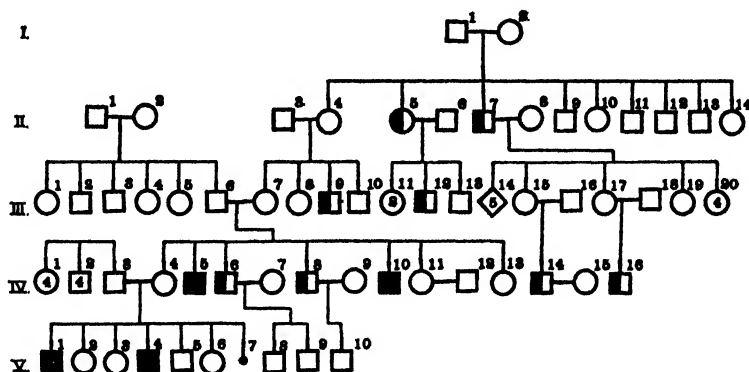


FIGURE 6.—Pedigree chart of the GROSS family in which both haemophilia and color-blindness occur. Color-blindness is indicated by the half-black symbol and haemophilia by the horizontal stripes. It will be noted that haemophilia is not found back of generation IV, while color-blindness occurs in generations II, III and IV. The two conditions are not combined in any one person.

III 7, the mother of the color-blind and haemophilic fraternity, stated that the haemophilic members of this family have always been recognized as such when only a few months old, in this respect differing from the MOLYNEUX family. Later they acquire painful swellings at the joints, originating even from only a very slight bruise.

III 7 has had 7 children, 4 boys and 3 girls. None of the girls were affected somatically, though the eldest has had 2 affected sons out of 3. Of III 7's children, IV 5 died in infancy from bleeding from a cut in the mouth made by a piece of glass.

IV 6 shows no symptoms of haemophilia. He was tested for color discrimination and was found to be color-blind.

IV 8 also shows no symptoms of haemophilia and is also color-blind. Thus both failed completely on the Stilling test group 2; also groups 4, 5, 6, 7 and 8, except that II 6 got 3 figures right out of 16, in the group 6 test. They made such errors on the group 9 test as to lead to the suspicion that they carried diminished susceptibility to red.

These two persons are the only ones in the immediate family, so far as studied, who showed color-blindness. In their mother's family color-blind persons (male only) occur as follows: Her brother, III 9; her aunt, II 5 (doubtful); this aunt's son, III 12; aunt's brother, II 7; and 2 of his daughters' sons, IV 13, IV 15.

There are no cases of color-blindness on the father's side of the house. Accordingly we may conclude that the gene for color-blindness was carried in the X-chromosome—as that is derived by sons from their mother's side only.

In the main fraternity there is one other male, brother of the color-blind men, who has haemophilia. IV 10, DANIEL G., is 28 years old, and had a bad spell about 3 months previously. These attacks resemble rheumatism, and have followed bad sprains of which he was, at the time, unaware. At other times he can do heavy work and not be troubled. ALFRED V 1 is the same way. When DANIEL was in the army hospital he had his ear pricked and it bled so profusely that his shirt became covered with blood. This came as a surprise to the physicians who a short time previously found that a finger prick was not followed by excessive bleeding.

In the fifth generation are two children, V 1 and V 4 who have a history of bleeding. V 4 died recently from pneumonia and bleeding. V 1, at 19 years, is tall and anemic. At the time of Mr. GREEN's visit in August, 1927, he was just recovering from a strain of some kind and had been laid up for 3 or 4 weeks. All of the other children look healthy.

Color tests were applied to the mother (IV 4) of the fraternity described in the last paragraph. She made only a few errors until she came to group IX, 2. In this group of 4 characters she attempted only 1, and this was correct. She made only 1 error with group IX, 1 and one error in group X, 1 and 2. She apparently has a trace of blue-green blindness.

Color tests were applied to the four oldest members of the fraternity of the haemophilic boy (V 1). He himself has remarkably good color discrimination, much better than his mother's. He made only one error in reading the 30 plates. V 2 ♀ made only one slight error. V 3 ♀ made only the same single error that her sister did. V 5 ♂, apparently about 6 years

old, made 14 errors. Especially did he fail in group IX—the test for blue-yellow. As this age may be too early for entire color discrimination capacity to be functional one can not conclude that any of the children are color-blind.

B-family

This is a branch of the MOLYNEUX family, the connection being through I 2, who was evidently a conductor. She had 10 children, 6 sons and 4 daughters. Four of the sons had haemophilia. Of these sons II 8 alone survives at the age of 70 years. He seems well preserved and looks normal. He was about 10 years old when it was first discovered that he was a bleeder. Later he had a permanent tooth pulled and it bled so badly that his pillow would be soaked in blood. Cuts and bruises sometimes trouble him. From a bad bruise on the shin he was confined to bed for 3 or 4 weeks. With the Stilling plates his color discrimination appeared normal.

II 8 has had 5 daughters and 1 son, all without haemophilia. Two full sisters of II 8 have respectively 1 son out of 2 and 1 out of 3 who are haemophilic.

III 2, daughter of II 8, has 3 sons, all haemophilic and 1 daughter. She herself has no bleeding tendency. She made 8 errors in the Stilling plates, all in miscalling a 3 an 8.

All of her sons are, as stated, badly affected with bleeding. One of them plays football and his bruises are terrible to see. Cuts cause all of these boys trouble. IV 5 was seen. He is a fine, normal appearing boy, tall and large for his age. His color discrimination is good.

G-O family

This is a branch of the MOLYNEUX family. I 2 was a PARDOE, allied to that family. She has a son who has haemophilia; also a daughter, aged about 12 years.

III 1, R.G., at 23 years is well conditioned but somewhat pale. He has had 3 bad attacks of haemophilia: (1) At 10 years he bumped his forehead. After seeming to heal, the wound, on the ninth day, broke out again. He was confined to his bed on this occasion. (2) At about 15 years he cut his foot on glass. After the blood flow was staunched it broke out again after a week, or 10 days; but he did not have to go to bed on account of it. (3) About 2 months before the interview he had a lower molar pulled. He was laid up for about 2 weeks. Treatment with horse serum worked satisfactorily. The young man has often had to stop nose bleed by the

use of a calcium compound, perhaps calcium lactate. He has good color discrimination, as shown by use of the Stilling test.

Pol family

This is a Ukrainian family, living in Cleveland, Ohio. The father, I 1, is dead; the mother, I 2, is about 25 years old. She is not a bleeder and can not find, after inquiry, any other cases of bleeding in the family. She has good color discrimination, confusing only 3's for 8's.

Her eldest son is 10 years old; and looks pale. He has had 4 bad periods of bleeding and has been in the hospital for them. At the time of Mr. GREEN's visit he was in the hospital due to a cut under the tongue from a piece of iron. A vesicle of blood under the tongue, size of a bean, was cauterized and removed. He had 4 transfusions of blood from his mother during about 2 weeks, the amount of transfusion being about 100 to 300cc; and the infusion was temporarily successful. The boy was recovering.

By Stilling's plates the mother read all figures correctly except for calling five 3's eights. The son, NICHOLAS, could not read the figures of group IX. Also most of the figures at group VI, especially 1 and 4, could not be read. The sister, aged 9, made a few errors which may be due to her immaturity. It seems probable that NICHOLAS carries 2 sex-linked defects; haemophilia and color-blindness.

Car family

In this Cleveland family the mother was interviewed by telephone through Doctor C. W. WYCKOFF's cooperation. This mother, who is herself free from haemophilia, has 2 living sons and had 2 others who died in early infancy. The family has been traced back for 5 generations and no case of haemophilia found previously. The elder son was discovered to be a bleeder at 6 months. He has been repeatedly troubled with bleeding and has had 5 blood transfusions; clotting time before transfusion 9 minutes, after transfusion 6 minutes. There are swellings at the joints and blood extravasations; but the lad seems to be getting better. The younger son was also a bleeder. If he bit his tongue or lip, the hemorrhage would persist for days. Clotting time 9-1/2 minutes.

Nor family

This Cleveland family was not seen but the haemophilic son of about 6 years was known by Professor T. WINGATE TODD of the Department of Anatomy, WESTERN RESERVE UNIVERSITY and Doctor DAVIS of the CITY HOSPITAL to be haemophilic.

Nar family

This Cleveland family was visited by Mr. GREEN. The father (H.S.N.) says he bleeds badly—though not so much of late. The worst time he had was when his teeth were extracted, for there was great difficulty in stopping the flow of blood. Of the mother's family little is known.

There is a daughter, MARGARET, aged 8 years. When she was a baby she was diagnosed by Doctor T. B. SMITH as a case of haemophilia. She has never had swellings at the joints. Doctor C. W. WYCKOFF finds her blood-coagulation time to be 15 minutes, and the coagulation is then not typical. A tonsil operation having been decided on she was given calcium lactate, 10 grains 3 times a day for 1 month prior to the operation. Her coagulation time was reduced to 5 minutes. Despite every precaution in the way of ligation of vessels the operator had a great deal of trouble for 10 or 12 hours in controlling the hemorrhage.

There are 2 sons, aged 4 and 2 years. It is early to say whether or not they are haemophiles. Doctor WYCKOFF states that they bleed very easily and for a long time after minor injuries, but their joints do not show swellings or blood extravasations. No color tests were made on this family.

CROSSING OVER IN MAN

Since there are several known sex-linked characters in man it is natural to look for crossing over. Crossing over in the children might be found in cases where a female with at least two sex-linked genes was mated with the "normal" male. These conditions are not easy to meet, since sex-linked phenotypic traits are relatively uncommon in females. But the conductor female can be inferred from her relationship to affected males. It is possible that crossing over might be found when the male carries 2 sex-linked genes. But in view of the relative inactivity of the Y-chromosome in man, as in *Drosophila*, it is to be expected that in the human, as in *Drosophila*, there may be no crossing over in the male.

In Mr. GREEN's study an attempt was made to find a person, either man or woman, who bore two genes for recessive, sex-linked traits. Haemophilia and color-blindness seemed the most promising. The GROSS family, indeed, showed both traits—in males only. But in this family no individual showed both traits. In the GROSS pedigree, III 7 is the sister of a color-blind man and her mother has a color-blind brother and the mother's sister has a color-blind son. This incidence of color-blindness in near relatives supports the conclusion from her progeny that she carries the color-blindness in one X-chromosome. III 7 transmits haemophilia to half of her sons and, accordingly, we conclude that she carries the gene

for haemophilia in one X-chromosome. The X-chromosome carrying color-blindness and the X-chromosome carrying haemophilia are apparently not the same X-chromosome. One might hastily suppose that if III 7 got the X-chromosome with the color-blind gene from her mother's side, she got that carrying the gene for haemophilia from the father's side. But, if so, the father would be haemophilic. But SIMON GROSS (II 6), her father, was seen and there is no doubt that he is free from the bleeding tendency, for his blood clotted in 1 minute. (GRACE ALLEN: E.R.O., A 8102-25).

Accordingly we are forced to the conclusion that both genes for color-blindness and haemophilia are carried in the X-chromosome that the mother (III 7) received from her mother or else that this X-chromosome underwent mutation in the haemophilic gene in III 7. Had the haemo-

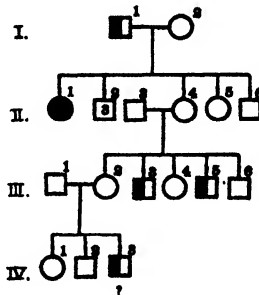


FIGURE 7.—Pedigree chart of a family with haemophilia combined with color-blindness (MADLENER 1928). I 1 both haemophilic and color-blind. II 1 (his daughter) is haemophilic III 3, 5 both haemophilic and color-blind. IV 3, certainly haemophilic and possibly color-blind.

philic gene been in the X-chromosome that III 7 received from her mother and which carried the color-blind gene we might expect haemophilia in III 7's brothers or her sister's sons, or those of her mother's sisters. III 7 had 2 brothers neither of whom was a haemophilic while 1 was color-blind. III 7 had 1 sister whose 2 sons were normal. III 7's mother had a color-blind brother but no haemophilic brother, out of 5 brothers. One sister had 2 sons, 1 color-blind. The other sister had 1 daughter who had 2 sons, not affected, and 3 daughters 1 of whom had 9 sons unaffected. The pedigree suggests that the haemophilia mutation had occurred in the paternal X-chromosome, while in the ovary of III 7.

The possibility that 2 sex-linked traits occur in the same individual has actually been realized in the remarkable German pedigree recently published by MADLENER (1928) which starts with one, H. DÖRR (figure 7). He (I 2) was a haemophilic and also color-blind. He had 7 children, of

whom 1 son was haemophilic and 1 daughter a conductor (to her 2 sons) of both haemophilia and color-blindness. In this case the sex-chromosomes of the mother of the 2 affected sons carried both a gene for haemophilia and one for color-blindness. Also a daughter of this mother certainly carried the gene for haemophilia and perhaps that of color-blindness (her 4 year old son is haemophilic but a little too young to be sure of his inability to distinguish colors). In this family, then, the linkage of 2 sex-chromosome genes is clear. Since the number of offspring is small it is not significant one way or the other that there is no evidence of "crossing over."

The pedigrees of AMMANN (1898) and of PFLÜGER (1881) published by BELL (1922) show no crossing over between night blindness and myopia and in the case of NETTLESHIP (BELL's figures 319-320 and 323) the incidence of these two traits is irregular. The cases of PAGENSTECHER (BELL, figure 318) and CUTLER (figure 325) are too fragmentary to permit of any conclusion as to the rate of crossing over.

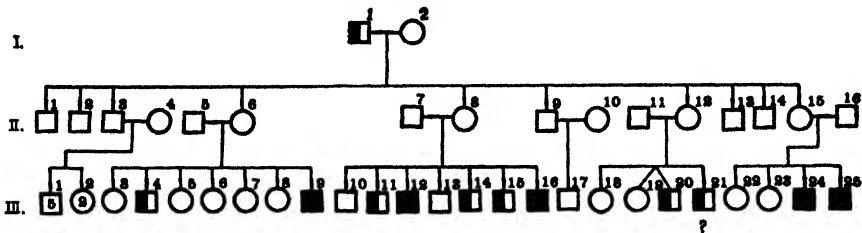


FIGURE 8.—Pedigree chart of a family combining night blindness and myopia (NEWMAN 1913). I 1 probably night blind and myopic. All symbols that are wholly black indicate night blindness without myopia; symbols that are half black indicate night blindness combined with myopia. There is doubt whether or not III 21 is myopic.

Instructive in the matter of crossing over is the pedigree given by NEWMAN (1913) and reproduced, in part, in figure 8. The significant individuals are described as follows:

I 1 at report 74 years old, night blind from infancy, "short sighted" (probably myopic). His six sons and four daughters are all normal. Of these children II 3 is not night blind or myopic and none of his 7 children are affected. II 6 is unaffected but is a conductor since both of her sons are night blind. III 4 "was unmistakably night blind and showed the usual associated defects" which the "Treasury of Human Inheritance" interprets as showing myopia. We have followed the "Treasury" in this interpretation. Thus the grandson had both the sex-linked traits of his grandfather. III 9 is typically night blind at 4 years. His sister, a university student of zoology, writes: "Brother can not see anything out of the light at night except that on bright nights he can see anything between

him and the sky." (Windmill, trees, houses, etc.) "He could not distinguish his express wagon, his kitten or other objects on the ground." "He is not short-sighted at all." In this case, then, only one of the two sex-linked defects was received by the night-blind son.

II 8 (Mrs. UZZELL) had 7 sons, III 10-16. III 10 is normal. III 11 is night blind, myopic and strabismic. III 12, at 13 years, is "night blind, without any other optic defect." Here again one of the sex-linked traits is lost. III 13 is normal. III 14 at 8 years is night blind, myopic and strabismic. III 15 at 6 years is likewise night blind, myopic and strabismic. III 16, at 4 years is night blind, but without other associated defects.

II 12, (Mrs. E. F. FLOYD) has had 2 sons, both night blind and 2 daughters normal. III 20, at 5 years, is clearly myopic and strabismic. The evidence is less clear that he is night blind.

III 21, now dead, "lived long enough to exhibit sure signs of night blindness. No facts about associated defects."

II 15 ♀ had 2 daughters and 2 sons. III 24, aged 4, is "night blind, but without other defects." III 25, aged 8 months at time of report, "shows unmistakable signs of night blindness, but no other defect." These two cases would seem too young for a critical statement.

To sum up, III 12, at 13 "night blind but without any other optic defect," a first cousin of the intelligent, trained reporter, is one clear case of inheritance of one of two genes linked in the mother's father. III 9, III 16 and III 24 are night blind but still too young for it to be certain that they are myopic. No case of myopia without night blindness occurs in this fraternity. However, the chance of failure to get just this condition is considerable.

That such a segregation of myopia from night blindness is possible is indicated in pedigree No. 325 of the "Treasury." In the latest generation of a night blind family one of 4 brothers shows night blindness and myopia. His eldest brother at 28 years had myopia of 9 D, but no night blindness. Their mother had 2 brothers. Of these 1 died at 9 years, night blind, but nothing is said about refraction. The other brother seen at 46 years was night-blind. His vision was poor but myopia is not mentioned. As the mother has a myopic (and night blind) nephew it seems highly probable that she carries both defects in one of her X-chromosomes and that crossing over has occurred in the gamete that went to form the eldest son.

We may conclude from these still incomplete facts and considerations that there is good evidence of crossing over in sex-linked characters of man. There is then in this phenomenon also no distinction between the genus *Homo*, *Drosophila* among insects and some flowering plants.

DISCUSSION

A review of sex-linked characters in man yields something of a surprise that in a species whose traits have been so long analyzed as man's there should be so few clear-cut sex-linked characters known. In *Drosophila* perhaps a hundred sex-linked characters have been determined. To be sure, there are only 4 pairs of chromosomes in *Drosophila* where there are 24 in man but even this fact does not account for the difference.

Another genetic difference between man and the insect is that while in the latter the sex-linked characters are mostly simple and clean-cut most of the sex-linked characters in man are so complex that their genetic behavior is not obvious. Red-green color-blindness alone seems fairly simple. Haemophilia is lethal when duplex and optic nerve atrophy, even if we eliminate the obviously non-genetic forms, shows many irregularities, such as the usual failure to reappear in the sons of daughters of affected men.

An interpretation of the confusing nature of sex-linked inheritance in man is to be sought, I conclude, in the same causes that are responsible for the generally small number of simple clean-cut genetic traits in man. Eye color and albinism, defects of eyes and appendages and Huntington's chorea among nervous diseases are the genetically simplest traits. Most traits, like pigmentation of skin and hair, aberrant proportions of body, and other bodily defects, mental traits of most sorts, and the susceptibility to many diseases, have a very complex genetic basis.

In general, it appears that man, perhaps just because he lies at the end of the most highly differentiated of evolutionary lines, has accumulated more mutations than any other species. Mutations have been added to preexisting mutations of the same organ. Also, it is probable that through translocations the same gene has been transferred, perhaps repeatedly, from one chromosome to another so that a gene is sex-linked in some strains and not in others. And, possibly through new relations to other genes, a trait that is recessive (or unexpressed in the heterozygote) in one strain becomes dominant (expressed in the heterozygote) in another. All of these possible complications have to be kept in mind in the study of human heredity. Probably in no other species is inheritance so complicated. This is not to suggest that the search for the inheritance factors in human traits has to be abandoned, but rather it has to be undertaken on a larger scale, with a greater expenditure of effort and funds. The importance to humanity of an understanding of the origin and development of human traits is so great, the issues at stake are so tremendous, that

research on a broader basis is urgently called for and would be wholly justified in its results.

NEED OF A REPOSITORY OF FAMILY HISTORIES

The study of sex-linked traits brings home very vividly to the geneticist the many difficulties in the study of human genetics. One of these is the time elapsing between the human generations. If the observer is a young man, of say 30 years, he may hope to observe the young children of a family, their parents and one or more grandparents, also collaterals. Such a young observer may, under fortunate conditions, see these children grow to maturity as he himself progresses toward old age. He may, before he dies, observe these children's children. Thus, under the most favorable conditions, he can see two complete mature generations; one incomplete one (of the grandparents) and one of immature individuals. For the study of inheritance of some characters this may suffice; but these favorable conditions are, alas, rarely realized. The observer wishes that, for his research, he had the contemporary physical and medical records of an earlier generation. But if any of them ever existed they have probably been destroyed. Then he regrets that there is no depository of medical and performance records about people. (as there is about race horses and cows).

In the case of sex-linked traits, where a generation is regularly skipped, and 2 or more may be, this need is especially acute. An example will illustrate this need. Suppose we have observed opticus atrophy in a young man of 20 years, and we desire to know if it be of the hereditary type or not. The parents, now 40 to 60 years of age, are not affected. Of the grandparents probably half are dead and any medical records about them have probably been destroyed. We must rely upon the testimony of the "parents" and their siblings, if any, as to the condition of sight in the grandparents, use of narcotics, diabetic symptoms, etc. The record we get from the parents will often be very incomplete. Were medical records extant, how eagerly would they be examined!

Or the case may be that of two parents both affected with opticus atrophy. At the time of observation they have children of 10 to 2 years. How important to know the history of these children 10 or 15 years later! Yet the observing physician may in the meantime have moved or the family may have found a home elsewhere and there is no one to follow up the family and record the subsequent history of the children. Were there a depository, in which the full record with names and addresses might be preserved, then the early history would not be lost and a student of hereditary opticus atrophy might complete the medical history of the family

and draw important biological conclusions. It ought to be regarded as against the public interest to destroy medical records. Individual interests are, of course, to be considered and safeguarded; but the interest of the race should be regarded as supreme.

SUMMARY

The different sex-linked traits show differences in genetic behavior. Red-green color-blindness behaves like a typical sex-linked trait. Haemophilia is exceptional in that all duplex recessive female zygotes die. Optic nerve atrophy appears in several genetic types, only one of which is typically sex-linked, and in that type daughters of affected males rarely have affected sons. Female conductors form a genetic stolon from which affected males bud off. Also the incidence of optic atrophy in females is unexpectedly high, probably due to the expression of the trait in the heterozygous condition.

Other sex-linked traits that are rare or less carefully analyzed are: hypoplasia of brain substance (MERZBACHER), night blindness (or hemeralopia), some strains of myopia, pseudohypertrophic muscular paralysis of GOWERS, and megalocornia. To these traits wanderlust or nomadism may be added. Still less well documented are coloboma, nystagmus, microphthalmia, ichthyosis, webbed toes, toothlessness and deficiency in sense of smell.

Since two sex-linked characters occurring in the same family should show linkage in inheritance such "double-recessives" were looked for. In a haemophilic family (GROSS) color-blindness was found, but the haemophilia appeared to be of such recent origin that linkage of its gene with that of color-blindness could not be studied. A family containing double recessive individuals (DÖRR) has been described. It is probable that the concurrence in some families of night blindness and myopia is due to sex-linkage. Crossing over between the genes of night blindness and myopia appears in the family described by NEWMAN, and more irregularly in a few others.

Finally the need of a repository of family histories to provide, in time, documentary evidence of traits of members of earlier generations—especially important in sex-linked traits—is stressed.

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TABLE 1

Incidence of hereditary optic atrophy in the offspring of various types of matings. N, normal; Con, conductor (heterozygous) female; Atrp, phaenotypically with optic atrophy.

MATING		SONS				DAUGHTERS			
FATHER	MOTHER	Number		Percent affected		Number		Percent affected	
		Total less † ^{yo}	Affected	found	expected	Total less † ^{yo}	affected	found	expected
1 N	Con.	419	263	63	50	328	26	8.9	0
2 N	Atrp	26	19	73	100	24	4	17	0
3 Atrp	N	86	5	5.8	0	72	2	2.8	0
4 Atrp	Con.	3	2	66	50	1	0	0	50
5 Atrp	Atrp	3	1	33	100	3*	0	0	100
		537	390	72.6		428	32	7.5	

* These daughters are still young!

† Died young.

TABLE 2a (continued)

REFERENCES	BOYS				DAUGHTERS				CONDUCTORS		Remarks	
	Reference to Mother	No.	X or $\frac{1}{2}w$	Affected		No.	X or $\frac{1}{2}w$	Affected		No.		Percent of fertile females
				No.	Percent			No.	Percent			
GOULD II (1893) WESTHOFF (1895)	I 2	3	0	2	67	2	0	0	0	g
	I 2	2	0	2	100	1	0	0	0	1	100	
	II 2	3	0	3	100	1	0	0	0	1	100	
	III 2	5		4	80	1	0	0	0	
	II 2	4	2	2	100	5	0	0	0	
SNELL (1897)	I 1	3	0	3	100	1	0	0	0	1	100	h
LEITNER (1897)	II 4	4	0	3	75		0					
LEITNER (1898)	I 2	3	0	0	0	9	0	0	0	3	33	
	II 1	3		3	100	3	0	0	0	
	II 2	4		1	25	3	0	0	0	
LEITNER (1898)	II 5	2		1	50	7	0	0	0	h
POSEY (1898)	II 7	2		1	50	3	0	0	0	1	33	
	III 3	1		1	100							
	I 2	1		1	100	1	0	0	0	1	100	
	II 1	3		1	33	2	0	0	0	2	100	
RAYMOND (1898)	III 1	2		1	50	1	0	0	0	h
	II 2	1		1	100	
	I 2	0		1	..	3				3	100	
	II 1	1		1	100	1	0	0	0	0	0	
	II 2	4		2	50	1	0	0	0	
HAWKES, (ERO) (1900 I)	II 3	2		1	50	1	0	0	0	h
	I 2	4	2	2	100	1	0	0	0	1	100	
	II 5	4		3	75	5	0	0	0	1	20	
	III 3	2		2	100	3	1	33	33	
	I 2	2		2	100	1	0	0	0	1	100	
HAWKES (1901 II)	II 2	4		4	100	1	0	0	0	1	100	h
	III 2	2		2	100	0				1	100	
		2		2	100	0				
	I 2	3		2	67	1	0	0	0	
		II 1	3		3	100					..	

TABLE 2a (continued)

REFERENCE NAMES	References to Mother	SONS				DAUGHTERS				CONDUCTORS		Remarks
		No.	X or 1/2	Affected		No.	X or 1/2	Affected		No.	Percent of fertile females	
				No.	Percent			No.	Percent			
LAEBER 1902-GUZMAN 1913 BRAMWELL (1903)	III 2	8	4	4	50	6	2	2	50	1	1	i
	I 2	3		1	33	2		0	0	2	100	
	II 2	3		1	33	2		0	0		..	
	II 3	2		1	50	5		0	0			
KOWALEWSKI (1906) USHER (1906)	II 2	3		2	67	1		1	100			j
	II 3	8		3	38	3		3	0			
	III 7	3		2	67							
	I 1	3		2	67	1		0	0	1	100	
CONTO (1907)	I 3	2		1	50							
	II 3	1		1	100	1		0	0			
	II 1	3		3	100	5		0	0	5	100	
	II 6	3		1	33	1		0	0		..	
HANCOCK (1908)	III 2	4		0	0	5		0	0	1	20	
	IV 1	1		1	100	0					..	
	III 3	4		1	25	2		0	0		..	
	III 4	3		3	100	4		0	0	0	0	
NETTLESHIP (1909, IV) (1909, VI) (1909, VII)	I 1	6		4	67	2		0	0	1	50	
	II 7	1		1	100	2		0	0		..	
	I 4	4		2	50	1		0	0		..	
	I 2	6		4	67	2		0	0		..	
EVANS (1909)	I 3	6	2	3	75	3		0	0	1	50	
	II 9	4	1	1	25	0		0	0		..	
	III 4	1	0	1	100	0		0	0		..	
	II 1	3	0	1	33	2		0	0	2	100	
BACH (1909) RAYMOND & KÖNIG (1909)	III 1	2	0	1	50	1		0	0		..	
	III 2	1	0	1	100	0		
	III 2	3		2	67	0					..	k
	III 2	5		3	60						..	
Mugge (1911, II) LUTZ (1911)	II 7	2	1	1	100	2	1	0	0		..	
	II 3	2		2	100	2				1	50	
	II 3	2		2	100							

TABLE 2a (continued)

REFERENCES	Reference to Mother	SONS				DAUGHTERS				CONDUCTORS		Remarks
		No.	X or 1/2	Affected		No.	X or 1/2	Affected		No.	Percent of fertile females	
				No.	Percent			No.	Percent			
TAYLOR & HOLMAN (1913 II)	0	3		1	33	4	1	0	0	2	67	
	I 2	6		4	67	4				0	0	
	I 5	3		3	100	4				1	33	
	I 1	2		1	50	3	0	0	0	2	67	
	II 3	0				2	0	0	0	2	100	
HENSON (1917 II)	II 5	0		4				2	50	
	III 1	2		0	100	0	0	0	0	
	III 2	3		1	33	0	0	
	III 7	2		1	50	3	0	0	0	
	III 9	2		1	50	1	0	0	0	
FLEISCHER & JOSENHAUS (1920)	I 2	2		1	50	3	0	0	0	3	100	
	II 3	4		0	0	2	0	0	0	1	50	
	II 4	3		2	67	3	0	0	0	2	67	
	III 4	2		2	100	4	0	0	0	3	75	
	III 10	3		0	0	3	0	0	0	1	25	
	III 15	3		2	67	2	0	0	0	1	50	
	IV 1	6		3	50	1	0	0	0	
	IV 2	5		2	40	6	0	0	0	
	IV 3	1		1	100	1	0	0	0	
	IV 16	4		2	50	0	
	IV 21	1		0	0	1	0	0	0	
	IV 27	5		1	20	3	0	0	0	
	II 7	3		2	67	2	0	0	0	
	I 2	7	1	5	84	4	0	0	0	3	75	
	II 2	6	2	3	75	6	1	1	20	
DuLEUTRA (1920) ERO MORLET, (1921) ERO	II 4	1	0	1	100	4	0	0	0	
	II 9	6	?	2	33	1	0	0	0	

TABLE 2a (continued)

REFERENCES	Reference to Mother	SONS			DAUGHTERS			CONDUCTORS		Remarks		
		No.	X or $\frac{1}{10}$	affected		No.	X or $\frac{1}{10}$	Affected				
				No.	Percent			No.	Percent			
VOGT & (KNÜSEL) 1922	II 1	2	0	2	100	4	1	0	0	3	100	1
	II 2	3	1	2	67	6	1	0	0	
	III 7	4	1	1	33	2	1	2	100	
	II 8	2	1	1	50	3	..	0	0	3	100	
	III 11	2	2	2	100	1	1	0	0	
GINZBURG (1923)	IV 25	2	1	1	50	2	1	0	0	
	IV 28	1	1	1	100	2	1	0	0	
	II 1	4	2	2	50	4	1	0	0	1	25	
	II 2	2	1	1	50	3	1	0	0	1	33	
	III 2	7	3	3	43	1	1	0	0	0	0	
WORTON (1913)	II 1	2	1	1	50	3	1	0	0	3	100	
	III 1	3	2	2	67	3	1	0	0	0	0	
	III 2	3	2	2	67	4	1	1	25	4	100	
	III 4	5	2	2	40	3	..	0	0	3	100	
	IV 7	3	1	1	33	0	..	0	0	
WAARDENBURG (1924)	IV 8	1	1	1	100	1	1	0	0	
	IV 9	1	1	1	100	1	1	0	0	
	IV 16	4	2	2	50	4	1	1	25	
	IV 20	2	1	1	50	0	..	0	0	
	IV 23	6	1	1	17	1	1	0	0	
MEYER-REMSLOH (1925)	IV 4	3	2	1	100	9	2	1	14	6	100	
	V 4	3	..	3	100	3	..	0	0	2	67	
	V 9	0	2	..	0	0	2	100	
	V 12	1	0	0	0	2	..	0	0	1	50	
	VI 4	3	2	2	67	3	..	0	0	2	67	
	VI 15	1	1	1	100	1	1	0	0	1	100	
	VI 16	1	1	1	100	0	..	0	0	
	VI 17	3	3	3	100	0	..	0	0	

TABLE 2a (continued)

REFERENCES	Reference to Mother	BOYS				DAUGHTERS			CONDUCTORS	
		No.	X or $\frac{1}{2}$ W	Affected		No	X or $\frac{1}{2}$ W	Affected		No.
				No.	Percent			No.	Percent	
MEYER-RIEMLSLOH (1925)	VI 19	1		0	0	3		1	33	..
	VI 20	2		2	100	7	1	0	0	1
	VI 22	4		1	25	0
	II 4	3		2	67	3	
KROPP (1927)	III 1	3		1	33	4		0	0	..
	III 3	2		1	50	3		0	0	..
USHER (1927)	III 7	2		1	50	7		0	0	..
	III 32	2		1	50	2		0	0	..
USHER (1927a)	III 34	3		1	30			0	0	
	III 35	1		1	100					
KAWAKAMI (1926)	III 10	4		3	75	6		0	0	..
	III 5	3		1	33	5		2	40	..
	II 4	1		1	100	1		0	0	..
	II 7	2		1	50	0	
	III 19	4		2	50	3		1	33	..
	III 26	3		2	67	5		2	40	..
Grand Total		444	25	263		345	17	26		

a. LEBER (1871) found daughter unaffected. SCHILLING (1875) found daughter affected but gives no ophthalmoscopic findings (DREXEL 1922, p. 54)

b. NETTLESHIP, 1909 p. clxii, figure 87. DREXEL 1922, p. 107.

c. The daughter has atrophic colored papillae; narrow vessels.

d. Other sibs?

e. Atrophic opticus.

f. Two other children, sex unknown.

g. DREXEL expresses doubt if typical Leber's disease; 3 cousins show white papillae.

h. Not all children of III 3 certainly known.

i. Other daughters affected left side, a central color section, papillae red gray. Not typical $\frac{1}{2}$; one daughter shows absolute central scotom; also temporal half papilla white.

j. Daughter shows central scotom and opticus atrophy.

k. Mother's mother's brother affected.

l. One of 2 daughters examined ophthalmoscopically.

TABLE 2b
Incidence of hereditary optic atrophy in progeny of specified matings. Mating II, F, normal; M, affected.

REFERENCES	Reference to Mother	SONS				DAUGHTERS				CONDUCTIONS		Remarks
		No.	X or typ	Affected		No.	X or typ	Affected		No	Percent of fertile females	
				No.	Percent			No.	Percent			
HASWELL (1888)	II 2	7		6	86	2		2	100	a
SYM (1891)	I 2	3		3	100	2		0	0	2	100	b
BACH (1909)	II 2	3		3	100	1		0	0	1	100	
WARDENBURG (1924)	IV 14	2		1	50							
MEYER-KRISLOH (1925)	V 8	3	1	2	100	4	2	0	0		..	
IRONSIDE (1926)	II 1	2	..	2	100	3	..	0	0		..	
USHER (1927)	III 22	0		..		1	..	0	0		..	
KAWAKAMI (1926)	II 11	2		0	0	2		0	0	1	50	
	II 12	2		1	50	4		2	50	2	50	
	III 30	1		0	0	3		0	0	0	0	
	III 31	2		1	50	4		2	50	
Grand Total		27		19		26		1				

a. No special age data in unaffected son; did he die before age of onset of disease.

b. Mother affected at 51 years (after menopause).

TABLE 2c (continued)

REFERENCES	References to Mother	SONS				DAUGHTERS				CONDUCTORS		Remarks
		No.	X or $\frac{1}{2}xy$	Affected		No.	X or $\frac{1}{2}xy$	Affected		No.	Percent of fertile females	
				No.	Percent			No.	Percent			
VOGT AND KNÜSEL (1922) GINSBERG (1923) HIRSCH (1923)	III 3	0	1	..	0	0	j
	III 5	2	..	0	0	0	
	III 7	3	0	3	100	0	
	III 7	2	0	0	0	4	..	0	0	
WORTON (1913)	III 8	1	0	0	0	2	0	0	0	k
	III 10	2	0	0	0	2	0	0	0	
	IV 25	1	0	0	0	2	0	0	0	
	III 12	4	0	0	0	1	0	0	0	
	III 15	4	0	0	0	0	0	
	IV 3	0	2	0	0	0	
WAARDENBURG (1924, I)	IV 21	1	0	0	0	5	0	0	0	
	VI 3	1	0	0	0	0	
	VI 10	0	2	..	0	0	
	VI 11	0	2	..	0	0	
MEYER-RIENSLÖH (1925)	II 2	4	..	0	0	6	..	0	0	
	II 1	2	0	0	0	3	..	2	67	
	III 4	6	0	0	0	4	0	0	0	
	III 8	1	0	0	0	1	0	0	0	
IRONSIDE (1926) KROPP (1927) USHER (1927, II)	IV 92	2	0	0	0	1	0	0	0	
	III 56	5	0	0	0	1	0	0	0	
	II 5	1	1	0	0	1	0	0	0	0	0	
	III 17	2	0	0	0	2	0	0	0	0	0	
Grand Total		39		5		41		2				

a. Also 4, sex unknown N.

b. 5, N, sex unknown.

c. 4, N, sex unknown.

d. 3, N, sex unknown.

e. 5, N, sex unknown.

f. 7, N, sex unknown.

g. 14 children, all N.

h. Also some additional daughters, unaffected.

i. The father (I 1) showed onset at 25 and later recovery, like grandsons!

j. Father and all 3 sons examined ophthalmoscopically, whitened papillae.

k. "Diagnose von Lebersche familie Optikus atrophie nicht absolut sicher."

l. No grandchildren or other descendants affected.

TABLE 2d
Incidence of hereditary optic atrophy in progeny of specified matings. Mating IV. F, affected;
M, conductor.

REFERENCES	REFER- ENCE TO MOTHER	SONS				DAUGHTERS			
		No.	X or $\frac{1}{2}$ yg	Affected		No.	X or $\frac{1}{2}$ yg	Affected	
				No.	Percent			No.	Percent
KAWAKAMI 1926	II 9	3		2	67	1		0	0
Total		3		2		1		0	

TABLE 2e
Incidence of hereditary optic atrophy in progeny of specified matings. Mating V. F, affected; M, affected.

REFERENCES	Reference to Mother	SONS				DAUGHTERS				CONDUCTORS	
		No.	X or $\frac{1}{2}$ yg	Affected		No.	X or $\frac{1}{2}$ yg	Affected		No.	Percent of fertile females
				No.	Percent			No.	Percent		
WARDENBURG (1924)	V 26	3		1	33	3		0	0	0	
USHER (1927)	III 6	0				0				0	a
Total		3		1		0		0			

a. Children mostly under 21; daughters probably under 15 years.

THE CORRELATION BETWEEN THE SEX OF HUMAN SIBLINGS. I. THE CORRELATION IN THE GENERAL POPULATION*

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INTRODUCTION

Of recent years problems of sex—variously designated as sex determination, sex inheritance, sex linkage, etc.—have been extensively investigated by experimental methods. Interest in this field of experimentation has perhaps diverted attention from the possibilities of the application of statistical methods to certain problems and to certain kinds of data for which experimental methods as conventionally understood can not readily be used. Quite obviously the two methods of research are not mutually exclusive. Both may contribute to a more complete understanding of a complex problem.

The purpose of this paper is to consider the correlation between the sex of human siblings by means of methods which, as far as we are aware, have not heretofore been applied to this problem.

The sex of human offspring is generally assumed to be distributed by chance except for the fact that the proportion of male and female births is not equal. Let n_m be the number of male births and n_f be the number of female births in families of a total size of $n_m + n_f = n$. It is generally sup-

* Part of the cost of the accompanying tables is paid by the GALTON AND MENDEL MEMORIAL FUND.

posed that if $p_m = \Sigma(n_m)/\Sigma(n)$ be the probability of male births and $p_f = \Sigma(n_f)/\Sigma(n)$ be the probability of female births, the chances of the $s+1$ th birth in any given family being male will be p_m no matter what the sex of the s preceding children.

If this assumption be true, the distribution of the sexes in N families of constant size, n , should be given by the terms of the point binomial $N(p_m + p_f)^n$, with standard deviation of number of males (or of females) $\sqrt{np_m p_f}$. GEISSLER as early as 1889 recognized the importance of taking $p_m > p_f$ and gave $(p_m + p_f)^n$ for large series of German families. NEWCOMB (1904) also employed point binomials for representing the distribution of the number of males and females in families, but took $p_f = p_m$.

If the empirical distribution of number of males (or females) per family of size n is to be compared with the theoretical distribution as given by the point binomial with a view to determining whether the discrepancies between the two distributions are larger than those to be expected as the result of random sampling, some statistical criterion of goodness of fit must be employed. PEARSON'S χ^2 , P test (1900) and ELDERTON'S tables for testing goodness of fit (1924) were not available to GEISSLER, but FISHER (1925, pp. 69-71) has shown that in the case of GEISSLER'S families of eight children the value of P (the probability that deviations as large as or larger than those actually observed may be reasonably supposed to have arisen from random sampling, and not to be confused with p_m or p_f as defined above) is small and that the actual standard deviation is larger than the theoretical.

FISHER notes the possibility of the presence of identical twins influencing the variance, but while he finds this factor inadequate to explain the differences between the observed and the theoretical standard deviation, he does not pursue the point further. The obvious explanation is that the sex of the offspring of the same parents is not wholly independent, but correlated.

METHODS

Given a series of families each of n children, six procedures for determining whether the sex of the offspring is distributed at random among the siblings are theoretically available.

(1) The actual distribution of number of males (or females) per family of given size may be compared with the theoretical distribution provided by the point binomial, as indicated above.

(2) The statistical constants of the actual distribution of number of

males (or females) per family of given size may be compared with the constants for the theoretical distribution.

(3) The relationship between the sex of any two births each occupying a definite place, say the r th and the t th, may be determined by the use of the four-fold table

		Sex of t th child		
Sex of r th child		m	f	Total
	m	n_{mm}	n_{mf}	$n_{mm} + n_{mf}$
	f	n_{fm}	n_{ff}	$n_{fm} + n_{ff}$
		$n_{mm} + n_{fm}$	$n_{mf} + n_{ff}$	

and the calculation of the correlation by methods applicable to such tables.

The applicability of the method is limited by the fact that in general the sequences of the sexes of human siblings are not given in the data, but only the numbers of each sex.

(4) If there be n siblings per family and if each be used once as a first and once as a second member of a pair we may regard the family as constituting a class and may obtain a four-fold intra-class (HARRIS 1913) correlation table $\Sigma[n(n-1)]$ entries, where Σ denotes summation for the N available families.

Such four-fold tables for the sex of members of the same sibship may be readily formed by methods indicated in a paper on the correlation between the fates of seeds planted in the same hill (HARRIS and NESS 1928) or from the moments of the frequency distributions by formulae given elsewhere (HARRIS, GUNSTAD and NESS 1930). The relationship between the sex of the children of the same family may then be expressed in terms of PEARSON'S equivalent probability correlation coefficient (1912).

(5) In cases in which the sex of any individual child, say the s th child, is definitely known and the total number of male and female children of the family is also available, the data may be represented in a bi-serial correlation surface, in which one variable is given in the alternative categories of male and female while the other is given in the quantitative terms of numbers of males or females in the $n-1$ remaining children of the family. Quite obviously such tables should be made for families of a given size, or precautions should be taken to express the number of males (or females) in the $n-1$ remaining children as ratios to $n-1$.

If the data permit this method of tabulation, it should be possible to

determine correlation coefficients by some modification of PEARSON's bi-serial method (1909).

(6) If the sex of the siblings of definite birth order be unknown but merely the total number of each sex in sibships of a given size, tables which are fundamentally symmetrical in nature but bi-serial in form may be constructed by determining the relationship between the sex of each of the n children classed in alternative categories and the sex of the remaining $n - 1$ children of families of constant size considered on the quantitative scale of number of males (or females) per $n - 1$ offspring.

Here, as in case (5), bi-serial theory must be applied.

These methods fall quite obviously into two groups. The first (methods 1 and 2) test for the existence of correlation between the sex of the members of the same family by determining the deviation of the number of either sex actually observed from the theoretical number, and expresses the closeness of agreement on an improbability scale. The methods of the second group (methods 3-6) express the differentiation of the families with respect to the tendency to produce an excess above the theoretical frequencies of children of either sex in terms of the correlation between the sex of the members of the same sibship.

No mathematical assumption concerning the nature of the "frequency distribution" of the character sex is necessary in the use of the first method. Given the probabilities p_m and p_f the point binomial is rigidly applicable as an expression of the theoretical distribution, on the assumption that the sex of the offspring is wholly uninfluenced by genetic or physiological variables peculiar to the family in which the offspring are produced. Practical limitations in the use of this method will be indicated below.

In the case of the four methods of the second group, certain underlying assumptions are necessary. Such assumptions are unavoidable when data can not be recorded on a quantitative scale. The methods of the group fall into two classes. The first involves a 2×2 -fold tabulation of the data, while the second involves a $2 \times n$ -fold arrangement of the data. These last two must be treated by the bi-serial theory. Since certain difficulties in the modification of the theory to adapt it fully to present needs are still to be overcome, these methods will not be further discussed in this paper.

The correlations based on four-fold tables must be determined by the classical four-fold r method of PEARSON, or by the newer equivalent probability method also proposed by PEARSON (1912). Since the first method assumes the normality of distribution of the two variables, it has seemed desirable to place our reliance on the equivalent probability method which

involves no assumption concerning the nature of the distribution. The calculation of the equivalent probability coefficient will be illustrated in greater detail below.

DATA ANALYZED AND RESULTS

The data here employed are drawn from the work of GEISSLER (1889), who has given records of the sex of 4,794,304 children. These are reported as born by 998,761 mothers, but these are weighted instead of actual numbers, since the full record of the mother and of her offspring is returned for each additional child reported during the ten year period 1876-1885. It must be noted that the series of families is not complete in that all cases in which only one child was born are omitted, and that the number of children is weighted, in that a mother who had borne two children before 1876 and who had borne three children during the decade would be recorded in families of three, four and five. These facts do not render the data unusable for present purposes, but it would be highly desirable to have the methods of analysis here developed applied to data for completed families—that is, to the records of numbers of children borne by mothers married for at least 20 or 25 years and having attained the age of 50 years.

Since GEISSLER's paper is relatively inaccessible we have rearranged his data to present the frequency distributions of number of males per family in the form of a correlation table between number of children born and number of males per family in table 1. All constants required in the present paper may be computed by the use of formulae to be given later from the data of this table.

Other series of data will be treated in a subsequent paper.

The frequency distribution of number of male children per family

The selection of the proper values of $p = \Sigma(n_m)/\Sigma(n)$ and $q = \Sigma(n_f)/\Sigma(n)$ to be used in calculating the terms of the point binomial presents no difficulty when only a single series of families of a given size is available, since the worker has no option but to use the actual numbers of each sex as given in his records. When families of different sizes are involved, the selection of the value of p requires some consideration.

GEISSLER determined the value of $p = 0.514768$ by taking the 2,468,305 males and the 2,325,999 females of his families of from 2 to 30 children and adding to them the 114,609 males and the 108,719 females born as the first child in the families of two children. Thus he based his value of p on the 2,582,914 males out of the total 5,017,632 weighted births. The values of p and q thus determined he applied to families of from 2 to 12 children.

Elsewhere we have shown (HARRIS and GUNSTAD 1930) that there may

be objection to GEISSLER's procedure in obtaining the number of males and females born in families of one child, but at this date our only alternative is to utilize the value $p = 0.514768$ as given by him for the whole series.

While on first consideration it might seem that the value of p' determined from all of the available data is the most suitable value to be employed in calculating the theoretical numbers of children of each sex in families of a given size (because of the fact that the errors of random sampling will be smaller for p' derived from the sample containing all the individuals than for the values of p derived from the sub-samples representing families of any given size) this is not perfectly clearly the case.

If the sex ratio changes with the size of the family (as has been suggested in the literature), the value of p will also change. If this be true the employment of one constant value of p for the whole series will result in the using of a value which is too low for certain ranges of size of family and too high for other ranges of number of children per family.

The alternative method of procedure is, of course, to determine the values of p independently from the data of families of each size.

As far as we are aware there is no *a priori* theory for deciding which of the two procedures is the more logical. All that can be done to settle the point is to determine whether the ratios of male births to total births changes significantly from smaller to larger families, or to express this same relationship in some other way. This problem has been investigated (HARRIS and GUNSTAD 1930) with the result that, for GEISSLER's data at least, there is a small and irregular but statistically significant increase in the proportion of males from the smaller to the larger sibships.

The results in the first section of table 2 are obtained from the constant value $p' = 0.514768$ while those in the second section are computed from the values for each individual family as given under the caption p .

The values of p tend to increase from the smaller to the larger families. Thus the values of $p - p'$ are negative for families of from 2 to 8 children and positive for families of from 9 to 18 children considered individually and for families of 19 to 30 children considered as a group. The methods of determining the ratios of $p - p'$ to their probable errors, $E_{(p-p')}$, and the significance of the differences have been discussed elsewhere (HARRIS and GUNSTAD 1930) and need not be considered here. For our present purposes the demonstration of a systematic trend in the values of p is of importance because it justifies the comparison of the actual numbers of children with two binomial distributions, one based on p and the other on p' .

The deviations of the empirical distributions of the number of males per family from the point binomials are expressed in terms of χ^2 and P .

Notwithstanding the fact that the point binomials are calculated in two ways, the values of P are low throughout. When the constant values of p' derived from all available data are employed the chances of the discrepancies between the observed and the theoretical distributions having arisen through random sampling are in all cases but one less than 2 in one hundred thousand. In 13 out of the 17 comparisons they are less than 1 in a million. When the value of p is determined independently for each size of family the highest value of P is only 0.0013 while 12 of the 17 values are less than $1/10^6$. In evaluating these results for the series as a whole it must not be forgotten that there is a certain, and unknown, amount of weighting due to two or more inclusions of some individuals. Since the distributions are calculated independently for each size of family, this limitation can not apply to the individual values of χ^2 and P .

Before closing this section a limitation of this method must be noted. As the size of the family increases the frequencies are distributed among an increasing number of classes. Thus there is a tendency for the values of χ^2 to become abnormally large through the influence of two purely statistical factors. First, the actual numbers of children must be recorded in integers whereas the theoretical numbers as given by the binomial may be given in fractions. Thus the ratio of the squared deviations of the observed from the theoretical numbers to the theoretical numbers may be abnormally large. Second, in the largest families, one or more classes may have no empirical frequencies. In such cases the class contributes the theoretical number of males to the value of χ^2 . It is for this reason that the point binomial has not been computed for families of over 18 children. For families of this size the values of χ^2 are unquestionably too high.

Expression of deviation of observed from theoretical distribution in terms of difference in variability

An alternative method of expressing the results is to compare the squared standard deviation (the variance) of the empirical and theoretical distributions with regard to their probable error. The theoretical variance for number of males per family for families of size n is

$$\sigma'^2 = \mu_2' = npq.$$

We require to compare the observed squared standard deviation, μ_2 with the theoretical by evaluating $(\mu_2 - \mu_2') \pm E(\mu_2 - \mu_2')$. Since μ_2' represents a theoretical distribution to be used as a basis of comparison we take its probable error to be 0, and determine

$$(\mu_2 \pm \mu_2') \pm E_{\mu_2}.$$

As is well known (PEARSON 1903)

$$E_{\mu_1} = 0.67449 \frac{\mu_1' - \mu_1'^2}{N}$$

where for samples containing two alternative classes only

$$\mu_2' = npq$$

$$\mu_1' = 3n^2p^2q^2 + npq(1 - 6pq)$$

and

$$\mu_1' - \mu_2'^2 = 2n^2p^2q^2 + npq(1 - 6pq).$$

These are easily evaluated. The results are given in table 3.

Here the first two columns give the size of the family and the total number of families in each class. The third, fourth and fifth columns give the empirical (μ_2) and the theoretical (μ_2') values of the second moment coefficients and the differences. The sixth column gives the ratio of ($\mu_2 - \mu_2'$) to its probable error. The final column gives $1/2(1 - \alpha)$ or the probability of deviations as large as or larger than those actually observed having arisen through the errors of random sampling.

Since SHEPPARD'S tables of the probability integral (1902) do not give the values of deviation $> 6\sigma$, we have had recourse to PEARSON'S (1912) table vi giving $-\log F$, where $F = \frac{1}{2}(1 - \alpha)$ as defined by Sheppard.

Without exception the values of $\mu_2 - \mu_2'$ are positive. Since in the table of the probability integral,

$$\frac{1}{2}(1 - \alpha) = \int_x^\infty z dx$$

gives the probabilities of deviation of the proportion of males as large as or larger than those actually observed having arisen through the errors of random sampling, a glance at the values in the final column of table 3 shows that the chances of obtaining through the errors of random sampling differences between μ_2 and μ_2' as large as those actually found are in general exceedingly small.

*The correlation between the sex of two consecutive children
of the same mother*

In only one case do GEISSLER'S (1889) data permit the determination of the correlation between the sex of two successive children of the same mother. This is possible in the case of families of two children. The frequencies for the 223,328 families and the routine of the correlation of the equivalent probability correlation coefficient are as follows.

		Second Child		
First Child		Male	Female	Totals
	Male	n_{mm} 59518	n_{mf} 55091	114609
	Female	n_{fm} 55196	n_{ff} 53523	108719
	Totals	114714	108614	223328

$$\chi^2 = \frac{(n_{mm}n_{ff} - n_{mf}n_{fm})^2 \cdot N}{(n_{mm} + n_{fm})(n_{mf} + n_{ff})(n_{mm} + n_{mf})(n_{fm} + n_{ff})}$$

$$= \frac{(144779078)^2 \times 223328}{(12460175871)(12459546396)} = 30.1529$$

$$\frac{1}{2}(1 + \alpha_1) = \frac{n_{mm} + n_{mf}}{N} = \frac{114609}{223328} = 0.513$$

$$\frac{1}{2}(1 + \alpha_2) = \frac{n_{mm} + n_{fm}}{N} = \frac{114714}{223328} = 0.514.$$

Interpolating from PEARSON's table V,

$$\chi_{\alpha_1} = 1.2536 \quad \chi_{\alpha_2} = 1.2537$$

$${}_{0}\sigma_r = \frac{1}{\sqrt{N}}\chi_{\alpha_1}\chi_{\alpha_2} = \frac{(1.2536)(1.2537)}{\sqrt{223328}} = \frac{1.57163832}{472.5759} = 0.00333$$

$$r_p^2(=) {}_{0}\sigma_r^2\chi^2 = (0.0000110889)(30.1529) = 0.00033436$$

$$r_p(=) + 0.0183.$$

The equivalent probability intra-class correlation between the sex of children of the same family

In the determination of the intra-class equivalent probability correlation coefficients the first task is the formation of the 2×2 -fold table. This is accomplished by use of the method of an earlier paper (HARRIS, GUNSTAD and NESS 1930) as follows:

Let n_m be the number of male births, and n_f the number of female births in families of total size $n_m + n_f = n$. Then the frequencies of the 4-fold table

	Male	Female	Totals .
Male	n_{mm}	n_{mf}	$n_{mm} + n_{mf}$
Female	n_{fm}	n_{ff}	$n_{fm} + n_{ff}$
Totals	$n_{mm} + n_{fm}$	$n_{mf} + n_{ff}$	$\Sigma[n(n-1)]$

may be written in terms of the first and second moments, and product moments of the variables.

$$n_{mm} = \Sigma[n_m(n_m - 1)] = \Sigma(n_m^2) - \Sigma(n_m)$$

$$n_{ff} = \Sigma[n_f(n_f - 1)] = \Sigma(n_f^2) - \Sigma(n_f)$$

$$n_{mf} = n_{fm} = \Sigma(n_f n_m) = \frac{1}{2}[\Sigma(n^2) - \Sigma(n_m^2) - \Sigma(n_f^2)].$$

Applying these formulae to the determination of the 2×2 -fold table for the relationship between the sex of the members of 95,390 families of 6 children each, for which the frequency distribution of number of males is given in table 1, we obtain the distribution shown in the following table

		Sex of "second" child		
Sex of "first" child		Male	Female	Totals
	Male	n_{mm} 758214	n_{mf} 712561	1470775
	Female	n_{fm} 712561	n_{ff} 678364	1390925
	Totals	1470775	1390925	2861700

The equivalent probability intra-class correlation may be determined (with a notation differing slightly from that used by PEARSON) as follows:

$$\chi^2 = \frac{(n_{mm} n_{ff} - n_{mf}^2) \cdot N}{[(n_{mm} + n_{mf})(n_{fm} + n_{ff})]^2} = \frac{(6601903175)^2 \times 2861700}{(2045737716875)^2} = 29.8032$$

$$\frac{1}{2}(1 + \alpha_1) = \frac{1}{2}(1 + \alpha_2) = \frac{n_{mm} + n_{mf}}{N} = \frac{1470775}{2861700} = 0.514.$$

Interpolating from PEARSON's table V,

$$\chi_{\alpha_1} = \chi_{\alpha_2} = 1.2537$$

$$\sigma_r = \frac{1}{\sqrt{N}} \chi_{\alpha_1, \chi_{\alpha_2}} = \frac{1.571764}{1691.6560} = 0.00093$$

$$r_p^2(=)_{\sigma_r^2 \cdot \chi^2} = (0.0000008649)(29.8032) = 0.00002578$$

$$r_p(=) + 0.0051$$

All of the data may be treated by the intra-class equivalent probability method. We have applied this procedure to individual families of 2 to 18 children, but have grouped together the results for the 141 families with 19 to 30 children, since the maximum number of any one of these classes is 77 for families of 19 children.

Table 4 gives the number of children per family, the frequency of each class of families, the entries (n_{mm} , n_{mf} , n_{fm} , and n_{ff}) of the four cells of the four-fold table, the value of χ^2 measuring the divergence between the actual frequencies and the theoretical frequencies of the four-fold distribution, and the equivalent probability correlation coefficients.

The correlation coefficients are of a very low order of magnitude, ranging from 0.0015 to 0.0824 but are positive in sign throughout. Since if there were no correlation between the sex of members of the same sibship the coefficients should be distributed about 0 with approximately equal numbers of positive and negative coefficients, we may note that if we ignore the magnitudes of the coefficients and consider only their uniform positive sign, we have about the same chance of obtaining these results by random sampling as of obtaining 18 consecutive heads in throwing a coin. This should occur about 4 times in a million.

The average values for the 17 coefficients for families of from 2 to 18 children is 0.0136. The coefficient for the 141 families with from 19 to 30 children is 0.0824. Combining all the data into a single four-fold distribution we find a coefficient of 0.0095.

The coefficient for the series as a whole is far lower than that for the families with from 19 to 30 children. It is quite probable that these very large families contain a relatively large proportion of twin births, many of which would be of the same sex. The low values for the series as a whole is determined by the enormous excess of records of families with small numbers of children. If we limit our attention to families of less than 9 children (for each class of which over 50,000 records are available) we find that the coefficients range from +0.0015 to +0.0182.

It is quite clear, therefore, that the sex of the members of the same family is not independent but correlated. Thus, certain parents have a definite tendency to produce families with a slight excess of males and

others to produce sibships with a slight excess of females, both beyond their theoretical frequencies as determined by laws of chance.

DISCUSSION, SUMMARY AND CONCLUSIONS

This paper, which is one of a series on the sex of human offspring, presents the results of an investigation of the distribution of the two sexes in large series of German families of various sizes and of the correlation between the sex of members of the same sibship.

It is shown that the distribution of the numbers of the two sexes in families of a given size is not strictly in accord with the theory that the sex of human offspring is determined wholly by chance, but that there is a significant deviation from a chance distribution.

Methods of expressing this deviation on the mentally comprehensible correlation scale are suggested. It is shown that when this is expressed in terms of intra-class equivalent probability correlation there is a very low positive relationship, of the order $r = 0.01$, between the sex of members of the same sibship.

With respect to the relative value of the two methods of analysis here suggested we may note the following considerations.

On first thought the comparison of the actual number of males or females in families with a given number of children with the theoretical number as given by the point binomial would seem to give the most critical and valuable criterion. This would seem to be true for two reasons. First, the assumptions underlying the application of these methods are of the simplest, most fundamental, and admittedly sound kind. Second, each frequency of the empirical distribution is compared with a corresponding frequency of the point binomial.

Against this procedure two objections must be urged. First, the χ^2 , P test becomes less reliable as the ratio of the number of classes to the number of individuals included in the empirical series becomes larger. Second, the values of P are often so nearly infinitesimal that they are mentally incomprehensible and non-comparable from series to series. These defects are apparently both eliminated by expression of the results in terms of equivalent probability intra-class correlation.

In the present case the results obtained by the two methods are wholly consistent as far as the biological generalizations to which they lead are concerned and differ only in the form and numerical values of the expressions. The uniform consistency of results for families of all sizes rather than the ratios of the individual correlation coefficients to their probable errors provides the basis for confidence in the statistical significance of the results.

As an incidental result of the present investigation we may note that it establishes empirically the significance of small values of the correlation coefficient.

Biologists often assert that correlation coefficients of the order $r=0.10$, $r=0.20$, etc. are "insignificant," "meaningless," "of no value," or "worthless." This is in part due to the fact that some biologists have not yet grasped the idea that statistical constants are to be judged by their ratios to their probable error and not by their absolute magnitude. It is in part due to the failure of most biologists to realize that with extensive data and refined methods of analysis biology may be a highly exact science, and that in consequence relationships of a very low order may be expressed with a high degree of confidence as to their validity.

In our opinion hypotheses as to the biological interpretation of these findings as to matters of fact should be held in abeyance until further quantitative evidence of various kinds is available. A suggestion that immediately presents itself is that the series of data, while primarily composed of single births, contains a certain proportion of twins and presumably a small proportion of triplets. These are generally known to show an abnormally high proportion of individuals of the same sex. Thus an attempt might be made to explain the present correlation as due to the mixture of highly correlated and uncorrelated materials. A discussion of the influence of the presence of these multiple births on the correlations must be reserved for a subsequent paper.

Finally we must emphasize the fact that sex in man presents a highly complicated problem or group of problems. The sex of all zygotes can not be ascertained, but only the sex of those zygotes which develop to an age of several months. Thus the investigation of the problem of prenatal mortality is necessary to a full interpretation of the present statistical results. Here, as in many other fields of biological investigation, individual series of data generally fail to provide all the information required. We must be content to postpone synthetic work until we shall have available a sufficient range of constants derived from various sources to make sound conclusions possible.

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TABLE 1
Distribution of numbers of males per sibship in GEISSLER's series of 998761 (weighted) families.

Number of Males	223328	179892	148903	120137	95390	72069	51680	38495	26500	16759	10690	6115	3332	1769	913	439	209	77	36	12	8	4	1	1	1	1	998761
21																				1		1					1
20																					1						1
18																						1					1
17																					1	1					4
16																						1					7
15																					2						13
14																					...						25
13																					...						105
12																					2						244
11																					3	1					595
10																					1	1					1557
9																					3	1					3606
8																							1				8086
7																											17031
6																											33985
5																											61679
4																											107327
3																											172683
2																											262103
1																											240886
0																											88822
	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	27	28	29	30	

Number of Children per Family

TABLE 2

Values of χ^2 and P measuring the divergence of numbers of males from the numbers calculated from the point binomial on the basis of the value of p for individual families and on the basis of p' for the whole series.

SIZE OF FAMILIES	NUMBER OF FAMILIES	CONSTANT p'		p	VARIABLE p			
		χ^2	P		$p-p'$	$p-p'/E_{p-p'}$	χ^2	P
2	223328	33.4387	.000000	.513422	-.001346	2.79	30.1547	.000000
3	179892	17.3975	.000601	.514716	-.000052	0.12	17.3862	.000604
4	148903	31.9469	.000004	.513757	-.001011	2.46	29.4160	.000007
5	120137	29.4945	.000019	.514041	-.000727	1.78	28.1743	.000035
6	95390	53.0014	.000000	.513951	-.000817	1.94	51.9114	.000000
7	72069	46.7716	.000000	.514648	-.000120	0.27	46.7392	.000000
8	53680	91.8993	.000000	.514677	-.000091	0.18	91.8965	.000000
9	38495	84.5168	.000000	.515039	+.000271	0.49	84.2706	.000000
10	26500	78.8680	.000000	.517494	+.002726	4.27	71.7229	.000000
11	16759	68.5036	.000000	.516895	+.002127	2.75	65.1896	.000000
12	10690	92.8036	.000000	.516830	+.002062	2.21	100.0275	.000000
13	6115	80.4336	.000000	.519026	+.004258	3.58	74.5460	.000000
14	3332	136.8569	.000000	.520215	+.005447	3.50	125.2893	.000000
15	1769	75.6104	.000000	.521688	+.006920	3.35	91.6556	.000000
16	913	164.0801	.000000	.521495	+.006727	2.41	148.0915	.000000
17	439	56.7651	.000008	.535710	+.020942	5.36	40.5625	.001291
18	209	76.8111	.000000	.526581	+.011813	2.15	55.0391	.000038
19-30	141527866	+.013098	2.06

TABLE 3

Comparison of actual variance ($\mu_2 = \sigma^2$) of number of males per family with theoretical variance ($= npq$).

SIZE OF FAMILY	NUMBER OF FAMILIES	μ_2	μ_2'	$\mu_2 - \mu_2'$	$E(\mu_2 - \mu_2')$	$\frac{\mu_2 - \mu_2'}{E(\mu_2 - \mu_2')}$	$\frac{1}{2}(1 - \alpha)$
2	223328	.505447	.499640	+.005807	.000714	8.14	21×10^{-9}
3	179892	.757856	.749350	+.008506	.001377	6.18	15×10^{-6}
4	148903	1.008960	.999243	+.009717	.002139	4.54	11×10^{-4}
5	120137	1.253921	1.249014	+.004907	.003075	1.60	14×10^{-2}
6	95390	1.523017	1.498832	+.024185	.004226	5.72	57×10^{-6}
7	72069	1.790443	1.748498	+.041945	.005753	7.29	44×10^{-6}
8	53680	2.067417	1.998277	+.069140	.007697	8.98	67×10^{-11}
9	38495	2.370400	2.247964	+.122436	.010300	11.89	14×10^{-17}
10	26500	2.655890	2.496940	+.158950	.013882	11.45	52×10^{-16}
11	16759	2.957159	2.746860	+.210299	.019300	10.90	89×10^{-18}
12	10690	3.339853	2.996601	+.343252	.026472	12.97	1×10^{-18}
13	6115	3.633895	3.245294	+.388601	.038038	10.22	26×10^{-18}
14	3332	4.260543	3.494279	+.766264	.055649	13.77	71×10^{-20}
15	1769	4.409851	3.742944	+.666907	.082019	8.13	21×10^{-6}
16	913	4.836813	3.992607	+.844206	.122054	6.92	15×10^{-7}
17	439	4.965750	4.228322	+.737428	.186810	3.95	39×10^{-4}
18	209	5.742376	4.487282	+.1255094	.287780	4.36	16×10^{-4}

TABLE 4

Frequencies for 2×2-fold intra-class correlation tables, values of χ^2 derived from four-fold table, and equivalent probability coefficient (r_p) measuring the correlation between the sex of members of the same family.

SIZE OF FAMILIES	NUMBER OF FAMILIES	FREQUENCY OF 2×2-FOLD TABLE				χ^2	r_p
		n_{mm}	n_{mf}	n_{fm}	n_{ff}		
2	223328	119036	110287	110287	107046	60.3034	.0182
3	179892	287486	268074	268074	255718	34.7723	.0089
4	148903	473076	444924	444924	423912	18.7725	.0051
5	120137	635486	599622	599622	568010	2.3159	.0015
6	95390	758214	712561	712561	678364	29.8032	.0051
7	72069	804734	753052	753052	716060	48.3909	.0063
8	53680	800000	747161	747161	711758	73.4430	.0078
9	38495	739934	687570	687570	656566	128.4641	.0107
10	26500	642916	591308	591308	559468	119.3125	.0111
11	16759	496068	456822	456822	433778	108.0492	.0120
12	10690	380588	348701	348701	333090	153.0095	.0164
13	6115	259356	235764	235764	223056	94.9359	.0157
14	3332	166666	148805	148805	142148	172.5552	.0265
15	1769	102284	91518	91518	86170	60.1719	.0200
16	913	60362	53908	53908	50942	43.5393	.0222
17	439	34592	29376	29376	26064	14.1875	.0172
18	209	17996	15681	15681	14596	17.3119	.0259
19-30	141	15636	12737	12737	12804	148.0288	.0824
2-30	998761	6794430	6307871	6307871	5999550	927.5633	.0095

THE INHERITANCE OF DORMANCY AND PREMATURE GERMINATION IN MAIZE*

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INTRODUCTION

Seeds of maize and in fact those of the majority of spermatophytes normally do not germinate during their period of development. Dormancy during this period is as essential to the perpetuation of the species as the ability to germinate after development has been completed. In maize, however, it has been found that the presence in the germplasm of various genetic factors results in germination, before the seed is mature, in certain definite proportions of the seeds. This condition has been reported and described by LINDSTROM (1923), EYSTER (1924a, 1924b), and by the writer (MANGELSDORF 1923, 1926). In the paper last cited, evidence was presented to show that a number of genetic factors are involved in the inheritance of premature germination in maize; that these factors operate at various stages in the development of the seed and differ in some of their effects. All are alike, however, in forcing the seed to germinate before development has been completed and hence, under natural conditions, are ultimately lethal in effect.

The present paper represents further studies on the inheritance of

* Part of the cost of the accompanying tables is paid by the GALTON AND MENDEL MEMORIAL FUND.

several forms of premature germination previously reported; the verification of several hypotheses which had been tentatively presented; and a study of new types more recently discovered. The studies reported here were made at the CONNECTICUT EXPERIMENT STATION from 1921 to 1926 and at the TEXAS EXPERIMENT STATION from 1927 to 1929. Some of the stocks used were found to be unadapted to Texas conditions and rather than delay completion of the work until new stocks could be developed, most of the F_3 progenies were grown in New Haven, the self-pollinated ears being returned to Texas for classification. I take this opportunity of thanking Doctor D. F. JONES and Doctor W. R. SINGLETON for their kindness in growing and pollinating this F_3 material. I wish also to acknowledge the assistance of Mr. A. T. CHISHOLM in classifying some 54,000 individuals included in these studies.

ORIGIN AND DESCRIPTION OF CHARACTERS

The origin and description of a number of these characters has already been given in a previous paper (MANGELSDORF 1926); consequently only a brief resumé need be included here. The reader is referred to the above paper for further details.

*g*₁

This is the first type of premature germination found. It was first noted in the second generation of inbreeding of a strain of Gold Nugget flint corn. Germination begins when the seeds are in the late milk stage and is associated with complete lack of color in the endosperm and chlorophyll in the plumule, except in the absence of light, when the plumule develops a faint tinge of bluish green. This character is inherited as a simple Mendelian recessive.

*g*₂

This character was found in an inbred strain of Golden Bantam sweet corn. Germination begins when the seeds are in the dough stage. The endosperm color is slightly affected but the plumules are normal green. This character is associated with small seeds which are defective in appearance and it may be that the premature germination is merely a secondary effect of a factor for defective seeds, since early germination of some of the hereditary types of defective seeds is by no means uncommon.

*g*₃

This stock was obtained from Doctor E. W. LINDSTROM and is the same one reported by him in 1923. It originated in an inbred strain of

Golden Bantam in the third generation of inbreeding. Germination begins at a very early stage and is almost completely associated with absence of color in the endosperm and lack of chlorophyll in the plumule. The g_{e8} type can be distinguished from g_{e1} only when both develop in darkness in which case the former is completely albinitic while the latter shows a faint greenish tinge. It is inherited as a simple recessive.

g_{e4}

An open-pollinated ear of flint corn of the Longfellow type received from Mr. T. B. MACAULAY of Montreal, Canada, gave rise to this type. Germination begins in the hard dough stage. Chlorophyll development is not affected and the endosperm color is only slightly diluted. The character is inherited as a simple recessive.

g_{e5}

This type of premature germination first appeared in the F_2 endosperm generation of a cross between g_{e1} and g_{e3} . It has since appeared in this same cross when repeated and in another cross in which g_{e3} was used as one of the parents. This situation is difficult to explain as the g_{e3} stock has always segregated in a normal 3:1 ratio. In appearance, g_{e5} differs in no discernible details from g_{e3} . Germination begins in the early milk stage and is associated with complete absence of endosperm and chlorophyll color.

g_{e6} g_{e7}

This character is due to the expression of duplicate factors. It was found in ears of an F_1 hybrid of two inbred strains of Canada Flint, neither of which had ever shown premature germination. Germination begins at the late dough stage. The plumule is green while the endosperm color is diluted only in small areas of the seed usually adjacent to the embryo. This character is inherited in a 15:1 ratio when both factor pairs are heterozygous; in a 3:1 ratio when one pair is homozygous recessive, the other heterozygous.

g_{e8} g_{e9} g_{e10}

A cross of an inbred strain of Sanford White flint with an unknown yellow flint gave rise to this stock. Germinating seeds were found in the F_2 endosperm generation in the ratio of 8:1. This was tentatively explained as the result of duplicate factors in the same linkage group. Later studies indicate that triplicate factors are involved and that two of these are linked. Germination begins at a very late stage and has no effect on chlorophyll development and practically none on endosperm color.

g_{e11}

This type of premature germination was first noted in the F_2 endosperm generation of a cross between two inbred stocks segregating for defective seeds, d_{e6} and d_{e11} , which later proved to be genetically identical. One parent was an inbred strain of Clarage Dent obtained from Mr. M. T. MEYERS, the other a strain of Reid's Yellow Dent from Doctor J. R. HOLBERT. Germinating seeds had never been noted in either of the parents, and since these were being studied especially for endosperm defects, it seems improbable that premature germination could have occurred without being noted. This type of premature germination is very striking in appearance, both the seed and plumule showing a pink color. The character appears to be inherited as a simple recessive.

 $g_{e12} \ g_{e13} \ g_{e14} \ g_{e15}$

Ratios of 255:1 and 63:1 in several crosses indicate a set of quadruplicate factors all of which must be present in a homozygous recessive condition in order for premature germination to occur. Germination does not take place until the seed is practically mature and is later than in any of the other types described. The plumule seldom ruptures the pericarp and chlorophyll and endosperm color are not at all affected. The sprouting is so slight that the seeds must be rather carefully examined from the germ side in order to identify the recessives.

 g_{ex}

This character is tentatively designated g_{ex} because little is known concerning it and it may prove to be identical with one of the types already described. It appeared in the F_2 endosperm generation of a cross between g_{e1} and $g_{e6} \ g_{e7}$, and was inherited as a simple recessive. In appearance it resembles g_{e4} rather closely and the two may prove to be genetically identical.

Peculiar origin of germinating seeds

An unusual fact regarding the origin of a large proportion of the types of premature germination is that they did not appear in the first generation of inbreeding when recessive characters are usually brought to light. Seeds of g_{e1} were first noted in the second generation; g_{e3} was discovered, according to LINDSTROM (1923), in the third generation; g_{e6} appeared only when g_{e3} was crossed with other stocks; g_{e11} resulted from a cross between two inbred strains which had never segregated for germinating seeds; and g_{ex} arose in a cross of $g_{e1} \ g_{e6} \ g_{e7}$. In addition $g_{e6} \ g_{e7}$, $g_{e8} \ g_{e9} \ g_{e10}$, and $g_{e12} \ g_{e13}$

$g_{e14} g_{e15}$ were found in crosses, the parents of which had never shown these particular types of premature germination. These types, however, belong to a different category. They are the result of duplicate, triplicate, or quadruplicate factors and their occurrence in crosses is readily explained by assuming that the parents of the crosses were of such a genetic constitution that segregation could not occur. For example, the most simple explanation of the appearance of $g_{e6} g_{e7}$ is that one parent was of the constitution $g_{e6} g_{e6} G_{e7} G_{e7}$, the other parent $G_{e6} G_{e6} g_{e7} g_{e7}$. Neither parent would segregate, nor would the F_1 seeds. In the F_2 generation, however, segregation in the ratio of 15:1 would be expected.

Such an explanation, however, does not account for the appearance of types which are inherited as simple recessives, such as g_{e1} , g_{e3} , g_{e5} , g_{e11} and g_{ex} . They might be the result of recent mutations but, if this is true, it is certain that mutations which result in premature germination are considerably more frequent than those of any other type so far observed in maize.

RESULTS OF CROSSING VARIOUS TYPES OF PREMATURE GERMINATION

The ultimate proof that all the types of premature germination, described above, are genetically distinct, must rest on the results of crosses in which the stocks are combined in all possible combinations. This program has not yet been completed but the data from such crosses as have been made, together with evidence of another nature, indicate rather convincingly that at least fifteen factors are involved in the inheritance of premature germination. The evidence for this conclusion is presented in the following pages.

Method of making crosses

Perhaps a word should be said here regarding the method used in crossing the stocks. Since all these characters are lethal they can be perpetuated only in the heterozygous condition. When dormant seeds from a segregating ear are planted, part of the plants thus produced are heterozygous; the remainder are homozygous dormant, the proportion depending upon the number of heterozygous factor pairs involved in the expression of the recessive condition. There is no way of distinguishing the two genotypes until several weeks after pollination. Consequently, the practice has usually been to pollinate at random at least five plants of one parent with pollen collected at random from five or more plants of the other parent. This practically insures that heterozygous plants of both parents are included but renders it impossible to predict the proportions in which the

various types of segregating progenies should appear in F_2 of any particular cross.

Method of presenting data

In all cases where crosses have been made, the two parental types of premature germination have reappeared in the F_2 endosperm generation which is borne on F_1 plants. The data from this generation are presented in detail and there appears to be no necessity for including the data on inheritance of the same characters in the parental stocks. The latter are therefore omitted except in the case of g_{e11} which has not yet been used in crosses.

When several types of segregating progeny occur in F_2 the different progenies have been grouped according to the Mendelian ratio which they fit most closely. Such an arbitrary grouping may be subject to errors, as SIRKS (1926) has pointed out, and the chief object in presenting the F_2 data in detail is that of demonstrating how large a proportion of the progenies fit a certain ratio within the limits of sampling errors, but differ significantly from other ratios which occur in the same population.

The fact that the segregation in any given progeny fits a particular Mendelian ratio does not, of course, prove that this ratio is the true expression of the segregation. Data collected at random are almost certain to fit one of the many possible Mendelian ratios within the limits of sampling errors. The behavior of the F_3 progenies, however, should show definitely whether any F_2 progeny has been correctly classified. F_3 progenies have been grown from F_2 populations representative of almost every ratio which occurred.

In tabulating the F_3 data it has not been considered necessary to present the data from each progeny separately. The progenies have been grouped, by simple inspection, according to the ratios which they appear to fit most closely. Some of the progenies might fall into either of two groups almost equally well but in general the groups have been well-defined with little overlapping. In addition the "goodness of fit" of each array has been calculated and with few exceptions was found to be reasonably close. The F_3 data indicate that such F_2 progenies as were tested had been correctly classified with regard to Mendelian ratios.

Results in F_2

$$g_{e1} \times g_{e3}$$

Since both of these characters are simple recessives it would be expected that the F_2 endosperm generation should produce two types of segregating

progenies, some segregating 3:1 and others segregating 9:7. In a previous cross of these two characters (MANGELSDORF 1926) these two types reappeared in F_2 , but in addition a third group, segregating in a ratio of 27:37, also occurred. This is difficult to explain since both parental stocks had previously segregated in a ratio of 3:1. The cross was, therefore, repeated with the results shown in table 1. The situation is almost identical to that found previously. Three types of segregating progeny appeared and these grouped themselves definitely into 3:1, 9:7, and 27:37 ratios. We must conclude that three factors for premature germination are involved in this cross. These are apparently independent in their inheritance, no evidence of linkage being exhibited. The third factor is presumably the one which has already been designated as g_{es} . Its effects are practically identical to those of g_{es} .

Ratios of 3:1 and 9:7 are expected from this cross if two pairs of complementary factors are involved. Table 2 shows that these requirements

TABLE 1
Segregating F_2 progenies of the cross $g_{e1} \times g_{e2}$.

EAR NUMBER	GENETIC FACTORS	PROBABLE RATIO	TOTAL SEEDS	NUMBER GERMINATING	THEORETICAL NUMBER GERMINATING	DEVIATION
2986	$g_{e1}, g_{e2}, \text{ or } g_{es}$	3:1	259	63	64.8	-1.8 ± 4.7
2987	"	"	281	75	70.2	4.8 ± 4.9
2990	"	"	271	76	67.8	8.2 ± 4.8
2991	"	"	251	57	62.8	-5.8 ± 4.6
2993	"	"	268	67	67.0
2994	"	"	221	53	55.2	-2.2 ± 4.3
2995	"	"	192	42	48.0	-6.0 ± 4.1
Total			1743	433	435.8	-2.8 ± 12.2
2988	g_{e1} and g_{e2} or g_{es}	9:7	270	111	118.1	-7.1 ± 5.5
2996	"	"	199	84	87.3	-3.3 ± 4.7
2997*	"	"	284	125	124.3	$.7 \pm 5.6$
2988	"	"	203	89	88.8	$.2 \pm 4.8$
Total			956	409	418.2	-9.2 ± 10.4
2989+	g_{e1}, g_{e2}, g_{es}	27:37	291	166	168.2	-2.2 ± 5.7
2992	"	"	224	132	129.5	2.5 ± 5.0
Total			515	298	297.7	$.3 \pm 7.6$

* See table 12 for F_2 progenies of this ear.

+ See table 13 for F_2 progenies of this ear.

$$g_{e1} \times g_{e4}$$

have been met. Three ears proved to be segregating for g_{e1} , two ears for g_{e4} , and the remaining three ears for both characters. The di-hybrid ears all show a close approximation to a 9:7 ratio, indicating that the two factors are independent in their inheritance. It is noted that two ears segregating in an approximate ratio of 255:1 are included in table 2. These are discussed later.

TABLE 2
Segregating F_2 progenies of the cross $g_{e1} \times g_{e4}$.

EAR NUMBER	GENETIC FACTORS	PROBABLE RATIO	TOTAL SEEDS	NUMBER GERMINATING	THEORETICAL NUMBER GERMINATING	DEVIATION
2969	g_{e1}	3:1	303	71	75.8	-4.8 ± 5.1
2972	"	"	237	48	59.3	-11.3 ± 4.5
2977	"	"	249	68	62.3	5.7 ± 4.6
Total			789	187	197.3	-10.3 ± 8.2
2970	g_{e4}	3:1	342	86	85.5	$.5 \pm 5.4$
2978	"	"	269	78	67.2	10.8 ± 4.8
Total			611	164	152.7	11.3 ± 7.2
2971	g_{e1}, g_{e4}	9:7	279	130	122.1	7.9 ± 5.6
2973	"	"	221	91	96.7	-5.7 ± 5.0
2975	"	"	315	145	137.8	7.2 ± 5.9
Total			815	366	356.6	9.4 ± 9.6
2974*	$g_{e12}, g_{e13}, g_{e14}, g_{e15}$	255:1	268	1	1.05	$-.05 \pm .69$
2976	"	"	266	2	1.04	$.96 \pm .69$
Total			534	3	2.09	$.91 \pm .97$

* See table 18 for F_3 progenies of this ear.

$$g_{e1} \times g_{e6}g_{e7}$$

Since g_{e6} g_{e7} are duplicate factors which result in ratios of 15:1 when both pairs are heterozygous, this cross should produce three types of segregating progenies; 3:1, 15:1, and 45:19. The last is actually the tri-hybrid ratio 45:15:3:1 in which the three last terms are combined because the three phenotypes are so nearly alike that accurate classification

is difficult. Occasionally it has been possible to separate the g_{e1} and $g_{e6} g_{e7}$ seeds on the same ear but generally all germinating seeds have been grouped together into a single class.

The results of this cross are set forth in table 3. It is evident that all three types of segregating progenies have appeared and hence the two stocks are distinct in their factors for premature germination. The close agreement between the theoretical and actual ratios indicates further that these three factors are independently inherited.

TABLE 3
Segregating F_2 progenies of the cross $g_{e1} \times g_{e6} g_{e7}$.

EAR NUMBER	GENETIC FACTORS	PROBABLE RATIO	TOTAL SEEDS	NUMBER GERMINATING	THEORETICAL NUMBER GERMINATING	DEVIATION
2954	g_{e1}	3:1	185	48	46.2	1.8 ± 4.0
2955	"	"	256	58	64.0	-6.0 ± 4.7
2958	"	"	105	22	26.2	-4.2 ± 3.0
2964	"	"	272	70	68.0	2.0 ± 4.8
Total			818	198	204.5	-6.5 ± 8.4
2950	g_{e2}	3:1	284	72	71.0	1.0 ± 4.9
2957	"	"	281	74	70.2	3.8 ± 4.9
Total			565	146	141.2	4.8 ± 6.9
2949	$g_{e6} g_{e7}$	15:1	205	13	12.8	$.2 \pm 2.3$
2953	"	"	227	13	14.2	-1.2 ± 2.5
2961	"	"	132	10	8.3	1.7 ± 1.9
2962	"	"	129	12	8.1	3.9 ± 1.9
Total			693	48	43.3	4.7 ± 4.3
2948	g_{e1}, g_{e6}, g_{e7}	45:19	297	92	88.2	3.8 ± 5.3
2951	"	"	348	100	103.3	-3.3 ± 5.8
2956	"	"	169	57	50.2	6.8 ± 4.0
2960	"	"	33	12	9.8	2.2 ± 1.8
Total			847	261	251.5	9.5 ± 9.0
2947*	$g_{e1}, g_{e2}, g_{e6} g_{e7}$	135:121	233	114	110.2	3.8 ± 5.1
2952†	$g_{e12} g_{e13} g_{e14} g_{e15}$	255:1	281	1	1.10	$-.10 \pm .71$
2963	"	"	171	1	.67	$.33 \pm .55$
Total			452	2	1.77	$.23 \pm .89$

* See table 16 for F_2 progenies of this ear.

† See table 18 for F_2 progenies of this ear.

In addition to the expected ratios, three types of segregating progenies, which had not been anticipated, were encountered in this cross. Two ears were found to be segregating in a ratio of 3:1 for a character which obviously was not g_{e1} . The recessive seeds on these ears did not lack endosperm color as do seeds of g_{e1} , but resembled more nearly the recessive seeds of g_{e6} g_{e7} . This situation might be readily explained by assuming that part of the plants of the original g_{e1} stock were homozygous recessive for either the g_{e6} or g_{e7} factor pair. This explanation is not tenable, however, because of a second unexpected progeny, Ear No. 2947, which segregates in a ratio of 135:121. This ratio does not differ significantly from a 9:7 but the F_3 progenies presented later show beyond doubt that the segregation is not 9:7.

This cross, then, as did that between g_{e1} and g_{e3} , has resulted in the unexpected appearance of a new type of premature germination not previously observed in either parent. The new character is tentatively designated as g_{ex} until it can be studied further. In appearance it is practically identical to g_{e4} and may prove to be the same genetically.

TABLE 4
Segregating F_2 progenies of the cross $g_{e1} \times g_{e3}$.

EAR NUMBER	GENETIC FACTORS	PROBABLE RATIO	TOTAL SEEDS	NUMBER GERMINATING	THEORETICAL NUMBER GERMINATING	DEVIATION
3008	g_{e3}	3:1	216	52	54.0	-2.0 ± 4.3
3009	"	"	258	61	64.5	-3.5 ± 4.7
3012	"	"	194	61	48.5	12.5 ± 4.1
3016	"	"	300	76	75.0	1.0 ± 5.1
3017	"	"	186	43	46.5	-3.5 ± 4.0
3019	"	"	242	57	60.5	-3.5 ± 4.5
Total			1396	350	349.0	1.0 ± 10.9
3013	g_{e4}	3:1	259	71	64.8	6.2 ± 4.7
3014	"	"	142	42	35.5	6.5 ± 3.5
3015	"	"	252	63	63.0	..
3018	"	"	101	35	25.2	9.8 ± 2.9
3020	"	"	239	71	59.8	11.2 ± 4.5
3021	"	"	304	78	76.0	2.0 ± 5.1
Total			1297	360	324.3	35.7 ± 10.5
3010	$g_{e6} g_{e4}$	9:7	272	120	119.0	1.0 ± 5.5
3011	"	"	130	51	56.9	-5.9 ± 3.8
Total			402	171	175.9	-4.9 ± 6.7

This cross, too, has produced two ears segregating in a ratio of approximately 255:1. These are discussed later.

$$g_{e3} \times g_{e4}$$

Two types of segregating progenies, approximating 3:1 and 9:7 ratios, are expected from this cross. Both of these types have been obtained as shown in table 4. The ears segregating in a 3:1 ratio have been further subdivided into two groups, those segregating g_{e3} and g_{e4} . Such a separation is possible in this cross because of the marked phenotypical difference in the two characters.

The only significant deviation in the data is a slight excess of recessives in the six ears which segregate for g_{e4} . One of these ears shows a deviation of 3.4 times the error and the deviation for the group is also 3.4 times the error. With this exception the data are in close agreement with the theoretical ratios and indicate that g_{e3} and g_{e4} are genetically distinct and independently inherited.

TABLE 5

Segregating F_2 progenies of the cross $g_{e6} g_{e7} \times g_{e3}$.

EAR NUMBER	GENETIC FACTORS	PROBABLE RATIO	TOTAL SEEDS	NUMBER GERMINATING	THEORETICAL NUMBER GERMINATING	DEVIATION
3046	g_{e3}	3:1	198	44	49.5	-5.5 ± 4.1
3045	$g_{e6} g_{e7}$	15:1	230	13	14.4	-1.4 ± 2.5
3048*	"	"	268	13	16.8	-3.8 ± 2.7
3050	"	"	276	14	17.2	-3.2 ± 2.7
3052	"	"	257	11	16.1	-5.1 ± 2.6
3053	"	"	260	9	16.2	-7.2 ± 2.6
3056	"	"	268	15	16.8	-1.8 ± 2.7
Total			1559	75	97.4	-22.4 ± 6.4
3049	$g_{e3}, g_{e6} g_{e7}$	45:19	246	82	73.0	9.0 ± 4.8
3051	"	"	285	105	84.6	20.4 ± 5.2
3055	"	"	246	76	73.0	3.0 ± 4.8
3057	"	"	240	70	71.3	-1.3 ± 4.8
Total			1017	333	302.0	31.0 ± 9.8
3047	$g_{e3} g_{e2}$	9:7	243	99	106.3	-7.3 ± 5.2
3054	"	"	137	61	59.9	1.1 ± 3.9
Total			380	160	166.2	-6.2 ± 6.5

* See table 14 for F_2 progenies of this ear.

$$g_{e3} \times g_{e6} g_{e7}$$

The results of this cross should be similar to those from the cross $g_{e1} \times g_{e6} g_{e7}$ in producing Mendelian ratios of three types, 3:1, 15:1, and 45:19. One ear of the first, six of the second, and four of the third were found, as shown in table 5. These were identified not only by the proportion of recessives but also by phenotypical differences in the two types of premature germination. The data show only a fair agreement with the theoretical ratios. Each of the six ears segregating in a 15:1 ratio shows a slight but not significant deficiency of recessives, while the total deviation for the group is 3.5 times the error.

This excess in ears which are segregating for all three factors may indicate linkage between g_{e3} and one member of the $g_{e6} g_{e7}$ pair, especially

TABLE 6
Segregating F_2 progenies of the cross $g_{e3} \times g_{e6} g_{e7}$

EAR NUMBER	GENETIC FACTORS	PROBABLE RATIO	TOTAL SEEDS	NUMBER GERMINATING	THEORETICAL NUMBER GERMINATING	DEVIATION
3044A	g_{e4}	3:1	285	71	71.2	$-.2 \pm 4.9$
3030	$g_{e6} g_{e7}$	15:1	255	17	15.9	1.1 ± 2.6
3032	"	"	260	26	16.2	9.8 ± 2.6
3034	"	"	52	4	3.3	$.7 \pm 1.2$
3035	"	"	226	20	14.1	5.9 ± 2.5
3036	"	"	180	12	11.2	$.8 \pm 2.2$
3038	"	"	267	25	16.7	8.3 ± 2.7
3039	"	"	294	12	18.4	-6.4 ± 2.8
3040	"	"	259	20	16.2	3.8 ± 2.6
3043	"	"	269	20	16.8	3.2 ± 2.7
Total			2062	156	128.9	27.1 ± 7.4
3033*	$g_{e4}, g_{e6} g_{e7}$	45:19	262	87	77.8	9.2 ± 5.0
3037	"	"	92	27	27.3	$-.3 \pm 3.0$
3044B	"	"	212	68	62.9	5.1 ± 4.5
Total			566	182	168.0	14.0 ± 7.3
3031	$g_{e12} g_{e13} g_{e14}$	63:1	90	1	1.4	$-.4 \pm 0.8$
3041†	"	"	122	2	1.9	$.1 \pm 0.9$
3042	"	"	219	5	3.4	1.6 ± 1.2
Total			431	8	6.7	1.3 ± 1.7

* See table 15 for F_2 progenies of this ear.

† See table 17 for F_2 progenies of this ear.

in view of the fact that ears of the same cross, but segregating for either character alone, were deficient in recessives.

This cross has, like that of $g_{e1} \times g_{e3}$, yielded an unexpected type of ratio. Two ears are clearly segregating in a ratio of 9:7 and all of the recessives on these ears are of the g_{e3} type. Apparently the g_{e6} factor postulated in the cross of $g_{e1} \times g_{e3}$ is also present in this cross. Since both crosses have g_{e3} in common as one parent it seems very probable that this extra factor is in some way contributed by the g_{e3} stock.

$$g_{e4} \times g_{e6} \quad g_{e7}$$

This cross, like the one immediately preceding, should produce three types of segregating progenies; 3:1, 15:1, and 45:19. All of these occur and the data agree fairly well with the theoretical ratios (see table 6). In this case, however, there is a significant excess of recessives among the ears segregating 15:1, in contrast to a deficiency of about the same magnitude in the preceding cross. Three ears segregating in a ratio of 45:19 indicate that three distinct factors are involved in this cross and that these are independently inherited.

$$g_{e8} \quad g_{e9} \quad g_{e10}$$

In a previous paper (MANGELSDORF 1926) the writer recorded the segregation for premature germination on three ears, two of which segregated in a ratio of approximately 8:1, the third in a ratio of 41:1. It was suggested that the 8:1 ratio might be the result of linked duplicate factors with approximately 33.3 percent of crossing over, while the third ear might represent linked duplicate factors in the repulsion phase or a set of triplicate factors, two of which were linked with approximately 33.3 percent crossing over. In addition to making a slight arithmetical error in calculating the ratios that might be expected when F_2 progenies of this ear were grown, I neglected to point out that the possibility of Ear No. 2178 being the result of linked duplicate factors in the repulsion phase was very remote indeed, and that the hypothesis of triplicate factors with linkage between two members of the set was far more plausible. In this case the 8:1 ratios would also presumably be the result of triplicate factors, the independent factor pair being homozygous recessive. Such a combination, in the absence of linkage, should have given 15:1 ratios.

It was realized that such an interpretation could be no more than tentative since any conceivable ratio can be explained by assuming a sufficient number of factors with appropriate degrees of linkage between certain members of the set. If, however, the 8:1 ratios are the result of two linked

factors with crossing over of approximately 33.3 percent, then we should expect to find in F_2 the following types of progenies in the proportion shown:

1. 8 ears segregating 3:1
2. 8 ears segregating 8:1
3. 2 ears segregating 35:1
4. 14 ears not segregating.

Seventeen ears of the F_2 endosperm generation were obtained with the results shown in table 7. Six of these ears were segregating for germinating seeds and it is noted that these ears fall into two distinct groups with regard to the proportion of recessive seeds. All of them deviate by significant amounts from a 3:1 ratio and four deviate significantly from a 15:1 ratio. On the other hand four of the ears fit an 8:1 ratio rather closely and the remaining two approximate a 35:1 ratio fairly well. Both of these ratios should be expected if linked duplicate factors with 33.3 percent crossing over are involved. The 8:1 ratios represent the coupling phase; 35:1 ratios the repulsion phase. Ratios of 3:1, resulting from a homozygous recessive condition of one of the pairs of factors which are also expected in F_2 , did not occur. The distribution as a whole, however, is in fairly satisfactory agreement with expectation. In a population of

TABLE 7

F_2 progenies from an ear (Number 2179) segregating in an 8:1 ratio in F_2 . Theoretical results calculated on the basis of duplicate factors with 33.3 percent crossing over.

EAR NUMBER	EXPECTED RATIO	NUMBER OF PROGENIES		TOTAL SEEDS	NUMBER GERMINATING	THEORETICAL NUMBER GERMINATING	DEVIATION
		Theoretical	Actual				
	3:1	4.25	0				
3098	8:1			287	25	31.9	-6.9 ± 3.59
3100	"			126	16	14.0	2.0 ± 2.38
3099	"			225	30	25.0	5.0 ± 3.18
3095	"			115	19	12.8	6.2 ± 2.27
Total		4.25	4	753	90	83.6	6.4 ± 5.81
3096	35:1			208	3	5.8	-2.8 ± 1.60
3097	"			51	1	1.4	$-.4 \pm .79$
Total		1.06	2	259	4	7.2	-3.2 ± 1.78
Not segregating		7.44	11				

seventeen ears the four types of progeny are expected in ratio of 4.25:4.25:1.06:7.44. They are found in the ratio of 0:4:2:11. Applying the formula for goodness of fit we find that $X^2=6.78$ and $P>0.05$.

F_3 progenies from the ear which segregated in a ratio of 41:1 were also grown. If we assume that this ratio is the result of a set of triplicate factors with linkage and 33.3 percent crossing over between two members of the set, then the following types of progenies are expected in F_3 :

1. 16 ears segregating 3:1
2. 10 ears segregating 8:1
3. 16 ears segregating 15:1
4. 12 ears segregating 35:1
5. 3 ears segregating 71:1
6. 83 ears not segregating.

Unfortunately the plants grown from this ear were rather weak and only fifteen self-pollinated ears were obtained. Twelve of these did not segregate; the segregation of the remaining three is shown in table 8. With so small a number of segregating ears it is naturally impossible for all the expected types to occur. Yet the agreement with expectancy is fairly close, X^2 being 3.47 and $P>0.5$.

TABLE 8

Segregating F_3 progenies from an ear (Number 2178) segregating in a 41:1 ratio in F_2 .

EAR NUMBER	PROBABLE RATIO	TOTAL SEEDS	NUMBER GERMINATING	THEORETICAL NUMBER GERMINATING	DEVIATION
3081	8:1	196	25	21.8	3.2 ± 2.97
3082	15:1	167	10	10.4	$-.4 \pm 2.11$
3080	35:1	141	3	3.9	$-.9 \pm 1.32$
Total		504	38	36.1	1.9 ± 3.90

In lieu of a larger population the best proof that three factors instead of two are involved is the appearance of a progeny segregating in a ratio of 15:1. It would be impossible for such ears to occur if only two linked factors were involved because so long as both factor pairs remained heterozygous only ratios of 8:1 resulting from the coupling phase and ratios of 35:1 resulting from the repulsion phase could occur. If a third factor, not linked with the other two, were involved, however, ratios of 15:1 would be expected in every case that either of the linked factor pairs be-

came homozygous recessive. It may be noted that Ear No. 3082 in table 8 fits a 15:1 ratio very closely. Furthermore, the segregation in this ear deviates from an 8:1 ratio by an amount equal to 3.13 times the error and from a 35:1 ratio by 3.75 times the error. The occurrence of this ear is rather convincing evidence that three factors are involved in this family.

Still another test may be made to determine whether two or three factors are involved in this stock and whether the linked factor hypothesis explains the situation. If a progeny which segregated in a ratio of 8:1 is crossed with an unrelated stock, then the heterozygous hybrid should segregate in a ratio of 8:1 if linked duplicate factors are involved. No ratios other than this could possibly be produced. If three factors are involved, however, and two of these are linked, then only ratios approximately 35:1 could occur in F_2 . This ratio is identical with that produced by two factors with the same degree of linkage in the repulsion phase, but by crossing with a totally unrelated stock it is assumed that one of the parents carries none of the recessive factors for premature germination and, hence, the coupling phase is the only one possible.

To make this test an F_4 plant from Ear No. 3081 was crossed by Yellow Creole, a southern variety quite different from the New England stock. Approximately half of the ear was self-pollinated, the other half crossed, the two types of seed being separated by xenia. The segregation among the self-pollinated seeds was 39 dormant:7 germinating; a ratio of 5.6:1. The crossed seeds on the same ear were all dormant. From these, three non-segregating and two segregating ears were obtained in the next generation (F_2 endosperm). The segregation on the latter is shown in table 9. It is noted that the ratios in the two ears are practically alike, 27.5:1 and 28.9:1. Both show a significant deviation from an 8:1 ratio but approach a 35:1 ratio rather closely.

These facts, considered in connection with previous data, furnish rather

TABLE 9

Segregating F_2 progenies from a cross of Ear Number 3081 ($G_{e8} g_{e8} G_{e9} g_{e9} G_{e10} g_{e10}$) \times Yellow Creole ($G_{e8} G_{e8} G_{e9} G_{e9} G_{e10} G_{e10}$).

EAR NUMBER	PROBABLE RATIO	TOTAL SEEDS	NUMBER GERMINATING	THEORETICAL NUMBER GERMINATING	DEVIATION
5648	35:1	371	13	10.3	2.7 ± 2.13
5649	"	359	12	10.0	2.0 ± 2.10
Total		730	25	20.3	4.7 ± 2.99

convincing evidence that a set of triplicate factors is involved in this stock and that two of these, arbitrarily designated as g_{e8} and g_{e9} , are linked while the third, g_{e10} , is inherited independently of the other two. All must be present, however, in a homozygous recessive condition in order for premature germination to occur.

Although all calculations of theoretical numbers have been based on an assumption of 33.3 percent crossing over between g_{e8} and g_{e9} , a tabulation of all available data would indicate that the percentage is somewhat lower than this and is probably nearer 30 percent.

TABLE 10
Segregating progenies of g_{e11} stock.

EAR NUMBER	GENETIC FACTORS	TOTAL SEEDS	NUMBER GERMINATING	THEORETICAL NUMBER GERMINATING	DEVIATION
3205	g_{e11}	549	158	137.2	20.8 ± 6.8
3206	"	340	85	85.0	
3207	"	245	71	61.3	9.7 ± 4.6
3208	"	458	112	114.5	-2.5 ± 6.3
3209	"	524	132	131.0	1.0 ± 6.7
Total		2116	558	529.0	29.0 ± 13.4

g_{e11}

No crosses have yet been made with this stock. The character is reported as a distinct type of premature germination because it differs phenotypically in several respects from all other types. As noted in the preliminary description the plumules are pink in color and there is also a pink tinge in the endosperm. In no other types of premature germination has such a condition been found and it seems safe to assume that g_{e11} is genetically distinct from the other types of premature germination, which have been described. The data in table 10 show that this character is inherited as a simple recessive.

$G_{e12} \ g_{e13} \ g_{e14} \ g_{e15}$

In a study on the inheritance of defective seeds, another type of endosperm character in maize, (MANGELSDORF 1926) it was found that the ratios were subject to slight errors due to the occurrence of non-hereditary defectives of various types on almost every ear. That a similar situation might obtain in the case of premature germination was suggested by the

fact that one or two germinating seeds were occasionally found in ears ordinarily classed as non-segregating. Consequently, all seeds from "non-segregating" ears of the F_2 population were carefully examined with the result that one or more germinating seeds were found on seven ears from the crosses $g_{e1} \times g_{e4}$, $g_{e1} \times g_{e6} g_{e7}$ and $g_{e4} \times g_{e6} g_{e7}$. The segregation on these ears has already been shown in tables 2, 3, and 6. When the data on these ears are combined as in table 11 it is found that the segregation in three of the ears approaches a 63:1 ratio rather closely, while in the remaining four ears the data do not differ significantly from a ratio of 255:1.

TABLE 11

Ratios of 63:1 and 255:1 from "non-segregating" ears of various crosses.

EAR NUMBER	GENETIC FACTORS	PROBABLE RATIO	TOTAL SEEDS	NUMBER GERMINATING	THEORETICAL NUMBER GERMINATING	DEVIATION
3031	$g_{e12} g_{e13} g_{e14}$	63:1	90	1	1.4	$-.4 \pm 0.8$
3041	"	"	122	2	1.9	$.1 \pm 0.9$
3042	"	"	219	5	3.4	1.6 ± 1.2
Total			431	8	6.7	1.3 ± 1.7
2952	$g_{e12} g_{e13} g_{e14} g_{e15}$	255:1	281	1	1.10	$-.10 \pm .71$
2963	"	"	171	1	.67	$.33 \pm .55$
2974	"	"	268	1	1.05	$-.05 \pm .69$
2976	"	"	266	2	1.04	$.96 \pm .69$
Total			986	5	3.85	1.15 ± 1.32

Had only the latter ratios occurred, the matter should probably have been given no further thought. The average number of seeds on the ears of these three crosses is approximately 235, and if one germinating seed occurred as the result of accident or other causes, a ratio approximating 255:1 would be inevitable. The fact, however, that ratios approaching 63:1 also occurred, that all these segregating ears were found in crosses of the same three stocks and that the non-segregating ears from all other crosses were completely free of germinating seeds, suggested with considerable force that genetic factors might be involved in the occasional appearance of these germinating seeds. Paradoxical though it may seem, the fact that the data do not exactly fit 63:1 and 255:1 ratios but show an excess of recessives, may be regarded as additional evidence of genetic segregation in those ratios. With the ears comprising an average total of 235 seeds it would be almost inevitable, as a result of random sampling,

that some of the ears from heterozygous plants should show no recessive seeds. As these ears cannot be identified they are not included as segregating ears and their omission is a constant source of error which tends to raise the proportion of recessives among the ears classed as segregating. How great this error may be in the present case cannot be determined because no record was made of the number of seeds on the non-segregating ears. However, if their average number is the same as that of the segregating ears in the same family, that is, 235, then if one non-segregating, though heterozygous, ear is included the ratio becomes 243:1. If two ears are included the ratio becomes 290:1.

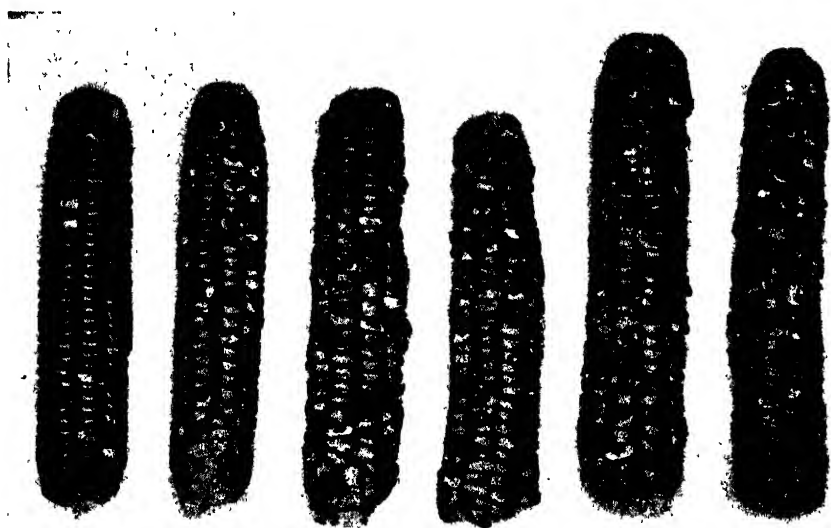


FIGURE 1.—Ears representing some of the ratios which occur in the inheritance of premature germination. From left to right the ratios are 15:1, 3:1, 45:19, 9:7, 135:121 and 27:37. Ears segregating 63:1, 255:1, and 225:31 were also found but were not photographed.

Ears segregating in a ratio of 63:1 are naturally subject to the same error though in a lesser degree. It may be of interest to note, in this connection, that NILSSON-EHLE (1909) in his study of the inheritance of seed coat color in wheat encountered precisely this error in a very marked degree. All of the 384 F_2 plants from a cross between red-seeded and white-seeded parents were red seeded. However, when 78 F_3 progenies were grown, ratios of 63:1, 15:1, and 3:1 were found. The failure of white-seeded plants to occur in F_2 appears to have been merely the result of random sampling, although the odds against the chance occurrence of this combination are approximately 75:1.

F₃ progenies from various F₂ ratios

In undertaking a study of segregation in F₃, it was obviously impossible, due to limitations of time and space, to grow F₃ progenies from any very large proportion of the F₂ ears, and it was considered more advisable to obtain F₃ progenies of the various ratios encountered in F₂ regardless of the parents involved rather than to grow F₃ progenies from each F₂ ear of any particular cross. In other words it seemed more important to learn, for example, how the F₃ progenies from an ear segregating in a 45:19 ratio in F₂ differed from those segregating 9:7 in F₂, than to attempt to prove that each ear which had been classified as segregating in a 9:7 ratio actually belonged in this category. Consequently, F₃ progenies have been grown from at least one ear representing each of the Mendelian ratios found in F₂ with the exception of the 3:1 ratio. The segregation for premature germination in various ratios is illustrated in figure 1.

Before proceeding with a discussion of the data it should be mentioned that classification in many of the F₃ progenies was subject to an unavoidable error as the result of accidental cross-pollination which inevitably reduces the proportion of recessives. This population came into bloom during a period of rainy weather and as a result many of the bags over the ear shoots were torn and the silks exposed for a brief period to cross-pollination. How great a percentage of cross-pollination occurred is impossible to estimate but the fact that many of the ears bore purple seeds as a result of pollination with other stocks in the vicinity is proof that it did occur. All purple out-crossed seeds were discarded in making the classifications but there were undoubtedly many other out-crossed seeds which could not be identified by color, and which, being classified as dormant, would tend to produce an excess of this class.

9:7 Ratio

Theoretically the dormant seeds from an ear segregating in a ratio of 9:7 should produce the following three types of progenies in the proportions shown:

1. 4 ears segregating 3:1
2. 4 ears segregating 9:7
3. 1 ear not segregating

An ear from the cross of $g_{.1} \times g_{.3}$ shown in table 1 was used to test this hypothesis. The results are shown in table 12. Thirty-five F₃ progenies were obtained. With this number the three types of progenies are expected

TABLE 12

F₂ progenies from Ear Number 2907 (9:7 ratio in F₂) G_{e1} g_{e1} G_{e2} g_{e2}.

EXPECTED RATIOS	NUMBER PROGENIES		TOTAL SEEDS	NUMBER GERMINATING	THEORETICAL NUMBER GERMINATING	DEVIATION
	Theoretical	Actual				
3:1	15.6	21	3295	757	823.8	-66.8 ± 16.8
9:7	15.6	14	2559	1027	1119.6	-92.6 ± 17.0
Not segregating	3.9	0	—	—	—	—
Total	35.1	35	5854	1784	1943.4	-159.4 ± 24.3

in a ratio of 15.6:15.6:3.9. They occurred in a ratio of 21:14:0. The agreement with expectancy is reasonably close, X^2 being 5.93 and $P > 0.05$. The recessive seeds in both the 3:1 and 9:7 groups show a marked deficiency which is undoubtedly due to contamination.

27:37 Ratio

Four types of progenies are expected from this ear in the proportions indicated.

1. 6 ears segregating 3:1
2. 12 ears segregating 9:7
3. 8 ears segregating 27:37
4. 1 ear not segregating

TABLE 13

F₂ progenies from Ear Number 2989 (27:37 ratio in F₂) G_{e1} g_{e1} G_{e2} g_{e2} G_{e3} g_{e3}.

EXPECTED RATIOS	NUMBER PROGENIES		TOTAL SEEDS	NUMBER GERMINATING	THEORETICAL NUMBER GERMINATING	DEVIATION
	Theoretical	Actual				
3:1	7.6	9	1549	382	387.3	-5.3 ± 11.5
9:7	15.1	16	2433	1021	1064.4	-43.3 ± 16.5
27:37	10.1	7	1221	659	705.9	-46.9 ± 11.6
Not segregating	1.3	2	—	—	—	—
Total	34.1	34	5203	2062	2157.6	-95.6 ± 24.0

Thirty-four progenies were obtained from Ear No. 2989, which had segregated in a ratio of 27:37 and was presumably heterozygous for g_{e1} , g_{e2} , and g_{e3} . With a population of this size the four types of progeny are expected in the ratio of 7.6:15.1:10.1:1.3. As shown in table 13, they oc-

curred in the ratio of 9:16:7:2. The results are in close agreement with expectancy, X^2 being 1.64 and $P > 0.50$.

15:1 Ratio

Progenies from plants segregating in a ratio of 15:1 should fall into three groups in the following proportions:

1. 4 ears segregating 3:1.
2. 4 ears segregating 15:1
3. 7 ears not segregating.

TABLE 14

F₂ progenies from Ear Number 3048 (15:1 ratio in F₂) G₆₆ g₆₆ G₆₇ g₆₇.

EXPECTED RATIOS	NUMBER PROGENIES		TOTAL SEEDS	NUMBER GERMINATING	THEORETICAL NUMBER GERMINATING	DEVIATION
	Theoretical	Actual				
3:1	8.5	7	1605	326	401.3	-75.3 ± 11.7
15:1	8.5	6	1188	40	74.2	-34.2 ± 5.7
Not segregating	14.9	19				
Total	31.9	32	2793	366	475.5	-109.5 ± 13.4

Thirty-two progenies were grown with the results shown in table 14. The theoretical distribution of 32 ears among these three groups is 8.5:8.5:14.9. The actual distribution is 7:6:19. The two distributions are in close agreement, X^2 being 2.14 and $P > 0.30$. In this case as in those already presented there is a deficiency of recessive seeds in both classes of segregating progeny, probably due to cross-pollination.

45:19 Ratio

A ratio of 45:19 is presumably the product of 3:1 and 15:1 ratios; one pair of complementary and a set of duplicate factors being involved. Consequently, five types of progenies are expected in F₂ in the following proportions:

1. 18 ears segregating 3:1
2. 4 ears segregating 15:1
3. 8 ears segregating 45:19
4. 8 ears segregating 9:7
5. 7 ears not segregating.

The 9:7 ratios are expected from plants that are heterozygous for the complementary factor and heterozygous for one pair of duplicate factors but homozygous recessive for the other pair.

TABLE 15

F₃ progenies from Ear Number 3033 (45:19 ratio in F₂) G₀₄ g₀₄ G₀₆ g₀₆ G₀₇ g₀₇.

EXPECTED RATIOS	NUMBER PROGENIES		TOTAL SEEDS	NUMBER GERMINATING	THEORETICAL NUMBER GERMINATING	DEVIATION
	Theoretical	Actual				
3:1	14.4	11	1989	480	497.3	-17.3 ± 13.1
15:1	3.2	5	722	50	45.1	4.9 ± 4.4
45:19	6.4	5	652	198	193.6	4.4 ± 7.9
9:7	6.4	7	1049	440	458.9	-18.9 ± 10.9
Not segregating	5.6	8	—	—	—	—
Total	36.0	36	4412	1168	1194.9	-26.9 ± 19.9

Thirty-six F₃ progenies were obtained with the results set forth in table 15. With a population of this size the five types of progeny are expected in a ratio of 14.4:3.2:6.4:6.4:5.6. They occurred in a ratio of 11.5:5:7:8. The agreement with expectation is very close, X² being 3.21 and P > 0.50. In this case the segregation within each group is also very close to expectation, there being no significant deviations.

135:121 Ratio

This ratio is presumably the product of 9:7 and 15:1 ratios. If this is true, four factor pairs are involved; two pairs of complementary factors and a set of duplicate factors. Seven classes of F₃ progenies are expected in the following proportions:

1. 32 ears segregating 3:1
2. 44 ears segregating 9:7
3. 16 ears segregating 27:37
4. 4 ears segregating 15:1
5. 16 ears segregating 45:19
6. 16 ears segregating 135:121
7. 7 ears not segregating.

Table 16 shows that with a population of 46 ears all the expected types of progenies, with the exception of those segregating in a ratio of 27:37, have occurred. With a population of this size the seven types are expected in a ratio of 10.9:15.0:5.5:1.4:5.5:5.5:2.4. They occurred in a ratio of 21:7:0:4:10:2:2. This array differs significantly from the theoretical,

χ^2 being 34.31 and $P < 0.01$. The segregation within each class, however, is in very close agreement with expectancy in every case.

TABLE 16
F₃ progenies from Ear Number 2947 (135:121 ratio in F₂) G_{e1} g_{e1} G_{es} g_{es} G_{o6} g_{o6} G_{o7} g_{o7}.

EXPECTED RATIOS	NUMBER PROGENIES		TOTAL SEEDS	NUMBER GERMINATING	THEORETICAL NUMBER GERMINATING	DEVIATION
	Theoretical	Actual				
3:1	10.9	21	3911	970	977.8	-7.8 ± 18.2
9:7	15.0	7	1087	459	475.5	-16.5 ± 11.0
27:37	5.5	0				
15:1	1.4	4	903	56	56.4	-0.4 ± 4.9
45:19	5.5	10	1926	595	571.8	23.2 ± 13.5
135:121	5.5	2	456	225	215.5	9.5 ± 7.2
Not segregating	2.4	2				
Total	46.2	46	8283	2305	2297.0	5.0 ± 27.5

Since a ratio of 135:121 seldom differs significantly from a 9:7 ratio, probably the best proof that the parent ear of these progenies segregated in the former ratio and not the latter is the appearance of 15:1 ratios in F₃. It would be impossible, as the result of recombination, for such a ratio to appear in the progenies of an ear segregating 9:7. The same holds true for the 45:19 ratios. The fact that both of these have appeared in F₃ is ample evidence that the F₂ ratio could not have been 9:7 in spite of the fact that the seven types of progeny have not appeared in the proportions expected from a ratio of 135:121.

63:1 and 255:1 Ratios

Ratios of 255:1 are presumably the result of a set of quadruplicate factors, all four factor pairs being heterozygous. The 63:1 ratios are assumed to be due to the same factor complex except that one of the four factor pairs is homozygous recessive. The latter should produce four types of progenies in the following proportions:

1. 6 ears segregating 3:1
2. 12 ears segregating 15:1
3. 8 ears segregating 63:1
4. 37 ears not segregating.

Twenty-eight F₃ progenies were obtained with the results shown in table 17. With a population of this size the four classes are expected in a ratio of 2.7:5.3:3.6:16.4. They occurred in the proportion of 0:2:3:23. Although no ears segregating in a ratio of 3:1 were obtained, the observed

array as a whole shows a reasonably close agreement with the theoretical array, X^2 being 7.52 and $P > 0.05$. The segregation within each class is also in agreement with expectation, the deviations being less than three times the error in both types of segregating progenies.

TABLE 17
F₃ progenies of Ear Number 3041 (63:1 ratios) G_{e12} g_{e12} G_{e13} g_{e13} G_{e14} g_{e14} G_{e15} g_{e15}.

EXPECTED RATIOS	NUMBER PROGENIES		TOTAL SEEDS	NUMBER GERMINATING	THEORETICAL NUMBER GERMINATING	DEVIATION
	Theoretical	Actual				
3:1	2.7	0				
15:1	5.3	2	431	18	26.9	-8.9 ± 3.39
63:1	3.6	3	505	7	7.9	$-.9 \pm 1.88$
Not segregating	16.4	23				
Total	28.0	28	936	25	34.8	-9.8 ± 3.92

From an ear segregating 255:1, the following F_3 progenies are expected:

1. 8 ears segregating 3:1
2. 24 ears segregating 15:1
3. 32 ears segregating 63:1
4. 16 ears segregating 255:1
5. 175 ears not segregating.

TABLE 18
F₃ progenies of Ears Numbers 2974 and 2952 (255:1 ratio) G_{e12} g_{e12} G_{e13} g_{e13} G_{e14} g_{e14} G_{e15} g_{e15}.

EXPECTED RATIOS	NUMBER PROGENIES		TOTAL SEEDS	NUMBER GERMINATING	THEORETICAL NUMBER GERMINATING	DEVIATION
	Theoretical	Actual				
3:1	2.3	1	216	23	54.0	-31.0 ± 4.29
15:1	7.0	4	482	28	30.1	-2.1 ± 3.58
63:1	9.3	6	1224	11	19.1	-8.1 ± 2.93
255:1	4.6	4	1005	4	3.9	0.1 ± 1.33
Not segregating	50.8	59				
Total	74.0	74	2927	66	107.1	-41.1 ± 6.86

F_3 progenies from two F_2 ears were grown and 74 self-pollinated ears were obtained. With this population the five classes are expected in the ratio of 2.3:7.0:9.3:4.6:50.8. Table 18 shows that they occurred in the proportions of 1:4:6:4:59. The agreement is reasonably close, X^2 being 4.60 and $P > 0.30$. It is noted, however, that the segregation within the

classes is not particularly close to expectation. The progenies segregating 3:1 and 15:1 both show a significant deficiency of recessive seeds. Progenies segregating in a 63:1 ratio also show a slight deficiency, while those segregating 255:1 are almost in perfect agreement with the theoretical ratio. In the latter case a slight excess of recessives would be expected, as has previously been pointed out, due to the exclusion of ears, which though heterozygous for all four factors pairs, produce no germinating seeds as the result of the operations of random sampling.

These consistent deficiencies of recessives in all four classes of segregating ears are probably due to contamination. Purple outcrossed seeds were particularly numerous in these progenies and other out-crossed seeds which could not be identified by color were probably just as numerous, or more so, as stocks with purple aleurone were further removed than other unrelated stocks with colorless aleurone.

In spite of this unfortunate error the evidence supporting a four factor hypothesis seems to be rather convincing. A consideration of the combined results of F_2 and F_3 leaves little doubt that the premature germination on these ears is inherited and that at least four recessive factors in a homozygous condition are necessary for premature germination to occur.

Although no crosses have been made between this stock and others which segregate for germinating seeds, it is not difficult to demonstrate that a different set of genetic factors is probably involved. It is fairly certain that the character is distinct from g_{e1} , g_{e2} , g_{e3} , g_{e4} , g_{e5} , and g_{e11} and because the latter are in each case governed by a single pair of complementary factors. The character is undoubtedly distinct from g_{e8} , g_{e9} , g_{e10} because in this set of triplicate factors two of the genes are apparently linked while the data on g_{e12} , g_{e13} , g_{e14} , g_{e15} indicate independent inheritance. Finally, this character is probably distinct from that produced by the duplicate factors g_{e6} , g_{e7} because several ears of the latter have been found which were also segregating for g_{e12} , g_{e13} , g_{e14} , g_{e15} . When these two types occurred on the same ear they could be readily separated, as the former produced a well developed plumule, while the latter showed only a slight elongation of the plumule beyond the region of the germ. To give but one example of the segregation of both types in the same ear, an F_3 progeny from Ear No. 2947 bore 273 dormant seeds, 22 seeds of the g_{e12-15} type and 26 seeds of the g_{e6-7} type. Apparently both types were segregating in a ratio of 15:1 so that the complete ratio would be 225:15:16. With a population of 321 seeds the theoretical distribution is 282:19:20. This agrees fairly well with the observed results, X^2 being 2.55 and $P > 0.20$.

Summary of segregation

Table 19 brings together in compact form practically all of the available data from each type of Mendelian ratio that has appeared in these studies. In making up this summary the data from tables 12 and 14 have been omitted because every class of segregating progeny included in these two tables shows a marked deficiency of recessives that cannot be attributed to chance and is undoubtedly due to accidental cross-pollination.

With the exception of the 9:7 and 45:19 ratios the data are in close agreement with expectation. No cause is known for the significant deficiency of recessives in the former and significant excess in the latter, although in the case of the 9:7 ratios part of the deficiency may be partly accounted for by cross-pollination.

TABLE 19

Summary of segregation classified according to Mendelian ratios.

FACTOR COMBINATIONS	THEORETICAL RATIOS	TOTAL SEEDS	NUMBER GERMINATING	THEORETICAL NUMBER GERMINATING	DEVIATION	DEV.
						P. E.
1 pair complementary	3:1	20430	5074	5108	-34 ± 41.7	.8
2 pairs complementary	9:7	10521	4420	4603	-183 ± 34.3	5.3
3 pairs complementary	27:37	2545	1444	1471	-27 ± 16.8	1.6
1 set duplicate	15:1	7873	491	492	-1 ± 14.5	.1
1 set triplicate	63:1	2160	26	34	-8 ± 3.9	2.0
1 set quadruplicate	255:1	1991	9	8	1 ± 1.9	.5
1 set duplicate:1 pair complementary	45:19	5008	1569	1487	82 ± 21.8	3.8
1 set duplicate:2 pairs complementary	135:121	689	339	326	13 ± 8.8	1.5
Totals		51217	13372	13529	-157 ± 67.3	2.3

Considering the population of 51,217 seeds as a whole, it is found that the total number of germinating seeds does not differ significantly from the theoretical number determined by totalling the calculated numbers for each class. The deviation is -157 ± 67.3 .

The percentage of recessives in this population has varied from 0.39 per cent in the 255:1 ratios to 57.81 percent in the 27:37 ratios, with intervening values ranging between these two extremes. The situation would have been hopelessly confusing had all these varying percentages appeared in a single population but occurring as they did in unrelated stocks, their analysis proved to be relatively simple.

These data furnish an excellent example of the tremendous complexity that may be expected from simple Mendelian combinations behaving in an orthodox fashion. When the disturbing effects of linkage and differential pollen-tube growth are also considered, it is evident that almost any percentage of recessives from 0-100 might occur. Even without these disturbing influences it is theoretically possible with the factors already studied to obtain a combination showing 92.49 percent of germinating seeds.

NUMBER OF DISTINCT FORMS OF PREMATURE GERMINATION.

The fifteen genetic factors for premature germination represent nine different types of premature germination. The evidence, though not complete, is sufficient to indicate that all of these are genetically distinct. That g_{e1} , g_{e3} and g_{e5} are genetically different is shown by the 27:37 ratios which are found when all three types occur in the same progeny. The marked phenotypical differences between g_{e4} and the types just mentioned are almost sufficient to prove that g_{e4} is different from the remaining three. In addition crosses of g_{e4} with g_{e1} and g_{e3} show the former to be genetically unlike the latter. The genotype g_{e2} has been crossed only with g_{e1} (MANGELSDORF 1926). Since the germination in g_{e2} is not always clear cut, this stock has been discarded, and the only evidence that it is different from the other stocks is of a morphological nature. This is also true of g_{e11} .

Because of the difference in mode of inheritance, it is almost certain that the genotype g_{e6} g_{e7} differs genetically from the five genotypes in which only one factor pair is involved. Crosses of g_{e6} g_{e7} with g_{e1} , g_{e3} and g_{e4} bear out this assumption, ratios of 45:19 having been observed in every case.

The genotype g_{e8} g_{e9} g_{e10} has not been tested in crosses. It is undoubtedly different from genotypes g_{e1} - g_{e6} because of the different mode of inheritance. Linkage of two members of this set of triplicate factors indicates that this genotype differs from g_{e6} g_{e7} and from g_{e12} g_{e13} g_{e14} g_{e15} , although there is a possibility that either, or both, of the latter may be homozygous recessive for an additional factor, which, if brought to light, would display linkage with one of those already known. This possibility is remote in the case g_{e6} g_{e7} as this character has been crossed with a number of unrelated stocks and no indication of additional factors has been found. The genotype g_{e12} g_{e13} g_{e14} g_{e15} has been shown to be distinct from g_{e6} g_{e7} by the segregation of both characters on the same ear. We may consider it, tentatively at least, to be different from g_{e8} g_{e9} g_{e10} because the latter exhibits linkage, the former not. The discovery of additional factors, however, in

either set might possibly show the two characters to be the result of the same factor combination.

With the exception of g_{e2} , and perhaps g_{e11} , the evidence is fairly conclusive in showing nine distinct types of premature germination, involving 15 genetic factors, to have occurred in the course of these studies.

DISCUSSION

The inheritance of premature germination is but another demonstration of the large number of genetic factors involved in a relatively simple phenomenon. Dormancy of the seed during development is so general that it may almost be taken for granted. Yet we find that at least fifteen different genetic factors are involved in maintaining dormancy and we can scarcely suppose that this is more than a sample of the total number.

As has been the case in several other characters in maize, two types of inheritance involving complementary and multiple factors, respectively, have been encountered. It is a temptation to assume that these two types of genetic phenomena are associated with distinct types of physiological reactions involved in maintaining dormancy. We might postulate, for example, that a series of complementary factors governs the formation of a group of substances, enzymes or inhibitors, all of which must be present in order to maintain dormancy, while a series of duplicate factors is responsible for another group of substances any of which will inhibit germination. In the first case the seed will germinate prematurely whenever any one of the essential inhibitors is lacking; in the latter case it will germinate only when all are lacking.

There is, at present, no evidence in support of such an interpretation. It may be more than a coincidence, however, that all the types of premature germination in which complementary factors are involved, occur relatively early in the development of the seed, while those in which multiple factors are involved occur relatively late. There are six characters in the former category and three in the latter. The test for independence shows the odds against the chance occurrence of this combination to be large, P being considerably less than 0.01. Perhaps these odds should not be taken too seriously as only nine characters are being considered and there is no sharp distinction between "early" and "late."

Since the discovery of duplicate factors and the introduction of the multiple factor hypothesis which was formulated independently by NILSSON-EHLE (1908) and EAST (1910), many cases of inheritance which fall into the category of duplicate factors have been recorded and there has

been no small amount of speculation regarding the origin of this hereditary mechanism.

The attractive, and very plausible, hypothesis of the origin of duplicate factors through doubling of the original chromosomes has met with several objections, two of which have been discussed in some detail by SHULL (1926). The first is that only certain characters exhibit the type of inheritance expected from duplicate factors, while other characters in the same race and even in the same linkage group are inherited as simple recessives. The second objection is that a doubling of the chromosomes would be expected to result, as MULLER (1914) pointed out some years ago, in ratios of 35:1 if the four homologous chromosomes assort at random. Ratios of 15:1 would not be obtained unless the four chromosomes should assort as two independent disomes rather than as a single tetrasome. SHULL (1926) states that deviations from a 15:1 ratio and approaching the tetrasomic ratio of 35:1 have occasionally been encountered in *Bursa*. In maize, DEMEREC (1923), working with albino seedlings, and the writer, in studies of premature germination, have observed ratios approaching 35:1. These have, however, been assumed to be the result of linkage of duplicate factors (repulsion phase) or linkage of two members of a set of triplicate factors. My own data from several generations fit the latter interpretation very well indeed, but there is always the possibility that additional studies will throw an entirely new light on the situation.

Probably a more serious objection to the chromosome duplication theory, at least as it applies to maize, is the occurrence of triplicate and quadruplicate factors. While these can be explained, as are duplicate factors involving two factor pairs, by additional doubling of the chromosomes, there is an arithmetical limit to the number of duplications which could have occurred to produce the present ten pairs. On the other hand it is by no means certain we have found the limit in the number of factors involved in the expression of a single simple character such as premature germination, although it is admittedly difficult to discover combinations larger than four factor pairs in population of the size ordinarily produced by single plants of maize.

If multiple factors have arisen through chromosome duplication, then the character g_{e12-15} which is the expression of four independent factor pairs accounts for four chromosomes, in the present complement of ten, which have originated from a single chromosome during the course of evolution of maize. If this were the case, perhaps we might expect that four of the present chromosomes would show considerable morphological

resemblance. A recent paper by McCLINTOCK (1929) indicates that this is not the case. Miss McCLINTOCK is convinced that every chromosome in maize is morphologically identifiable, differing from every other in some essential feature.

It is possible that the hypothetical transition from tetrasomic to disomic inheritance, that is, from a tetraploid to a "double diploid," has been accompanied by discernible morphological changes in chromosomes that were originally identical and homologous. The fact remains, however, that at present there is very little, if any, critical evidence that multiple factors arise through chromosome duplications. The hypothesis remains a plausible one, however, particularly in view of the fact that duplicate factors and polyploidy are both of frequent occurrence in plants and of rare occurrence in animals.

SIRKS (1926) suggests that some cases of inheritance attributed to duplicate factors are interpreted equally well by the molecular hypothesis of CORRENS (1919) which postulates that molecules in homologous chromosomes can exchange in either direction one or more of the atoms of which they are constituted. Thus, what appears to be a series of multiple factors might in reality be a series of multiple allelomorphs. SIRKS' argument was partly based on the assumption that it had never been shown conclusively that certain Mendelian factors were linked with one of several members of a series of multiple factors and inherited independently of other members of the series. He was not familiar at that time, however, with the work of WARREN (1924) on inheritance of egg size in *Drosophila* and LINDSTROM (1926) on the inheritance of size of fruit in the tomato. Both of these writers have definitely localized in certain linkage groups one or more of the genes of a series of multiple factors.

In view of these facts, SIRKS' argument loses much of its force. We must not overlook the possibility, however, of a more complex situation than simple re-combination of duplicate factors, particularly when the data show marked deviations from normal ratios, and appear, at first glance, to suggest linkage. Perhaps it is more than a coincidence that the ratios 8:1 and 27:1, which DEMEREC (1923) found in the case of white seedlings; the ratio of 10:1 which STEWART (1928) encountered in a study of inheritance of awns in several crosses of wheat; and the ratios of 7.5:1 and 35:1 in premature germination which I have recorded in this paper, are so closely alike. All have been interpreted on the basis of duplicate factors with linkage and crossing over of the order of 33 percent. This appears to be the most simple interpretation and is probably justified until more adequate linkage tests with other factors of the same linkage group, or data of another nature, show that it is no longer tenable.

SUMMARY

1. Premature germination is an inherited character in which the seeds fail to maintain dormancy during development and germinate before maturity.

2. This character has appeared in many different stocks of maize, in several cases when two stocks were crossed, and in others in the second and third generation of inbreeding.

3. At least fifteen different genetic factors and nine distinct characters are shown to be involved in the inheritance of premature germination. There are recognizable differences between some of the types in the time at which germination begins as well as in other phenotypical characteristics.

4. Six of the characters are the result of complementary factors and are inherited in ratios of 3:1, 9:7, and 27:37, depending on the number of factors involved in the cross.

5. Three characters are governed by duplicate, triplicate, or quadruplicate factors and are inherited in ratios of 15:1, 63:1, and 255:1.

6. Ratios of 8:1 and 35:1 are interpreted as a result of triplicate factors, two of which are linked with approximately 33 percent of crossing over.

7. Combinations of 3:1 and 15:1 ratios produce a ratio of 45:19. Combinations of 9:7 and 15:1 ratios result in a ratio of 135:121.

8. Practically all of the different Mendelian ratios encountered have been verified by the data from F_3 progenies.

9. The possible origin of duplicate factors through chromosome doubling is discussed in detail.

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ARE "PROGRESSIVE" MUTATIONS PRODUCED BY X-RAYS?¹

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I. THE PHYSICAL BACKGROUND OF THE PROBLEM

Do the X-ray mutations consist merely of losses and rearrangements of portions of the chromosomes? Or do they also include "progressive" changes—the kind of steps of which real evolutionary advancement has been composed? This is one of the most vital immediate questions confronting X-ray genetics, for upon the answer to it may depend in turn the answers to fundamental questions concerning the mechanism of natural evolution, the nature of the gene, and the means of bringing about artificial evolution. The issue, therefore, must be faced squarely.

¹ During the course of the radiation work in this laboratory both authors became interested in the problem in this paper and accumulated various data relative to it. It was then decided to present a combined report of all the data, and it was arranged that the senior author (PATTERSON) should conduct the major experiments described, those in sections IX to XIII inclusive. Except where otherwise specified, the other experiments described, and the discussion, are by the junior author.

There are two main facts which tend to give plausibility to the suspicion that X-rays may not be constructive in their genetic action. One is the general fact that they do often cause "destructive" changes (especially noticeable when the treatment is heavy or long continued) both in the case of organisms and of non-living materials. The other is the more specific fact, discovered coincidentally with the gene-mutation effect, that they cause breakages of chromosomes, sometimes accompanied by losses of the fragments and sometimes by their reattachment at a different point in the chromatin from before (MULLER 1927, WEINSTEIN 1928, MULLER and PAINTER 1929, PAINTER and MULLER 1929).

A little further inquiry into the manner in which X-rays exert their chemical effects serves to dispel the idea that the changes induced must always be of a destructive nature—no matter how we choose to define the word "destructive." At the same time it becomes evident why many of the changes so induced are judged to be destructive. The primary effect of the X-rays, as also of beta, gamma, and cosmic rays, on the structure of any material is the expulsion of an electron (sometimes more than one) from some of the atoms, either directly, by the absorption of a quantum of the radiant energy, or, in the case of far more of the atoms (some hundreds to one like the former), indirectly, through the electron's being forced out by another electron that was previously expelled through the (direct or indirect) action of the radiation. In case an electron is held by two atoms in common it may become detached from one of them, thus causing the molecule to become directly broken up. Sometimes, when the action is "indirect" in the sense above explained, instead of an electron's being completely ejected, it is merely forced to a position more distant from the atom nucleus. In the case of ejection, the atom remaining is usually incomplete or "ionized"; in the case of displacement of the electron, the atom is sometimes said to be "excited." No atoms are exempt from these effects. In any case, but especially in the case of ejection, the altered atom is in a state of higher potential energy than before, and of heightened reactivity. It is now often capable of releasing its former intra-molecular union with some other atom or atoms, and of forming a new union, the exact nature of which will depend upon what other atoms, and atom-groups, happen to present themselves in an appropriate manner.

Thus, in an organic mixture that is complex to start with, many different kinds of new compounds will come into existence in a chaotic fashion, lying in the form of isolated molecules scattered indiscriminately through the mixture. Of course any such hodgepodge of changes should in general make for disorganization and will thus be more and more "destructive" in

its effect, the stronger the treatment is, that is, the more multitudinous these random changes are. Nevertheless, many of the new molecules, individually, may be just as complicated, and some more complicated than the old. It is inevitable that many of the changes—though doubtless (because of the smaller number of chances for this) not the majority—should be of the nature of syntheses, and that a rather high proportion of the changes should be endothermic, involving intake of energy, as compared with the original substance, by virtue of the previous absorption of the energy of radiation.

There is no mechanism by which a gene, whatever its setting, could be protected from the occasional occurrence of such an individual molecular alteration when subjected to X-rays or related radiation. Mutant genes thus produced would therefore be expected to involve alterations of varied nature, not merely losses, breakages, and the sequelae of these (although it might well be the case that losses and partial or complete inactivations would be produced oftener than changes of other kinds).

It is true that not all the induced mutations need be supposed to result from any such immediate effects of the rays. It might be postulated that some particular substance, or physical condition, to which the genes were sensitive, was first produced in the protoplasm in some abundance, as a regular result of irradiation, and that this in turn then reacted upon the genes. If so, it might further be postulated that this latter action was a purely destructive one. There is now, however, an accumulating body of experimental evidence which militates against such an idea by tending rather to the conclusion that the induced mutations are in general the direct result of the local electronic "hits." These findings may be briefly passed in review here.

(1) There is, first, the general randomness in the occurrence of the induced mutations. (2) There is, more especially, the fact that, of two chemically identical allelomorphs present near together in a treated cell, only one becomes altered (p. 532-537; also MULLER 1928 a, c). (3) The degree of phaenotypic change (proportion of lethals to non-lethals; likelihood of the most extreme allelomorph being produced) is obviously independent of the strength of the treatment (MULLER 1928c, 1929a). (4) Attempts to produce gene mutations by the application of specific substances, without irradiation, have so far failed (MULLER 1923, 1928c, 1929a). (5) Most important of all, probably, is the fact that a direct and simple proportionality has been shown to exist between the frequency of the induced mutations and the amount (energy) of the radiation absorbed. This has

been proved by HANSON and HEYS (1929) for radium rays (beta, gamma, and mixed) and more recently by OLIVER (1930) for X-rays. There is no indication in the results of any lower critical intensity, or threshold value, beneath which there is no (or a relatively lesser) effect. All these facts and considerations converge to indicate strongly that the mutations are rather direct effects of individual quantum-absorptions and electron-hits, and that the changes involved in them are therefore probably most varied in their nature and rich in their possibilities.

II. EVIDENCE CONCERNING THE DUALITY OF THE X-RAY EFFECT ON CHROMATIN

What, now, is the bearing on the question at issue of the finding that X-rays produce not only such changes as would ordinarily be recognized as "gene-mutations" or "point mutations," but also clearly recognizable "chromosome abnormalities," which have obviously resulted from chromosome breakage, loss of pieces, and shifted attachments of pieces, and the further fact that these "abnormalities" are comparable in numbers, or perhaps even more numerous than the apparent "point mutations" (MULLER and ALTENBURG 1928, 1930)? Other things being equal, would it not be simpler to assume only one kind of effect rather than two or more radically different kinds of simultaneous effects, such as inter-genic rearrangements and intra-genic changes in composition would seem to be? Is it not for this reason probable that the apparent "point mutations" are really only smaller editions of the "chromosome abnormalities"?² Our tests for chromosome abnormalities are only (1) the cytological test (2) the finding of changed linkage values in previously known genes, and (3) the simultaneous "mutation," loss or duplication of several linked genes. It must certainly be admitted that pieces of a chromosome might at times become lost or displaced which were so small as to answer to none of these tests.

The argument concerning the question just raised will take two different forms, according to whether or not it is proposed that the natural mutations, as well as those artificially induced by radiation, consist merely of losses, attachments, and rearrangements of portions of chromosomes. This, however, is a thesis that can scarcely be seriously maintained nowadays, especially in view of our knowledge of the linear differentiation of the chromatin into hundreds, at least, of qualitatively distinct parts (the genes), and in view also of the now well-known objections to the

² This position has recently been stated in considerable detail by SEREBROVSKY (1928, 1929), and by DUBININ (1929).

theory of "presence and absence" as an explanation of all² Mendelian differences. It seems absurd to suppose that all the different genes now existing must in ages past have arisen, *de novo*, full-fledged, in their present form, from non-genic material, and that subsequent evolution has involved merely their loss, rearrangement, or change in proportionate numbers. There is no evidence forcing genetics into any such *cul-de-sac*.

On the contrary, it might with more reason be maintained that some mutations may consist of actual new "creations" of genes from non-genic protoplasmic material. It is conceivable that such an event occurred in the origination of bar eye, since the normal, non-bar, is merely the absence of bar (STURTEVANT 1925). The fact that infra-bar acts as if recessive to bar (WRIGHT 1929b) but not to normal genes at any other locus raises difficulties in supposing that bar arose by translocation or duplication of some other locus, and hence tends to support the interpretation of a *de novo* origin. If, however, an event of such a radical nature could be proved to have occurred, we should have even stronger grounds for supposing that new kinds of genes could also arise by the mere change in composition of a preexisting gene.

If, now, it be agreed that natural mutations include real changes in the inner composition of the genes, the most obvious objection to putting the X-ray mutations into a different category in this respect is the fact that they resemble the natural ones so closely. Those which may be classed as "visibles" give no more evidence of involving two or more contiguous loci (that is, of being losses of sections of a chromosome) than do the natural visible mutations. Many of them, in fact, are sensibly identical with well-known natural mutations, not only in phaenotypic effect, but also in locus and other genetic behavior. The clustering of the mutations in the genetic map of the X-chromosome (in which alone it has been studied adequately in the case of the induced mutations) is the same both in the case of the natural and the induced mutations (MULLER 1928b, HARRIS 1929). There is a similar excess of recessives over dominants found among both cases, natural and induced. The evidence so far indicates that the natural mutations which have been found to recur most often (for example, white eye, rudimentary wing) also recur with unusual frequency after irradiation.

² At the same time it is reasonable to suppose that some mutations involve losses even though in any given case we cannot determine whether or not a loss has occurred. It is even quite possible that a large proportion of mutations may consist of losses, or at least of inactivations,—a view recently redefended by WRIGHT (1929a), on the basis of the recessiveness of most mutations. This is a question which remains for future investigation, and which should not be confused with the question of whether *all* mutations are losses.

Thus a study of the induced "point mutations" themselves lends absolutely no support to their being viewed as different from the natural ones. It may further be observed that the apparent point mutations produced by X-rays are surprisingly abundant in comparison with the losses and displacements of large sections of the chromatin, if they represent only the extreme lower limit of size of the latter.

Especially noteworthy in the present connection is the fact that the proportion of the "point mutations" which are lethal (or otherwise deleterious) seems to be no higher in the case of the induced than of the natural mutations. Thus, in the first experiment in which mutations were produced by X-rays, there were in all 89 "point mutations" in the X-rayed chromosome; of these 57 were lethal, 14 were to be classed as "semi-lethals" (viability from 1 to 10 percent of the normal), and the rest, 18, were "visible" mutations of greater viability. With this there may be compared a previous experiment of MULLER and ALTENBURG (1919, 1920) on natural mutations in the X chromosome. Out of a total of 18 mutations in this experiment, there were 13 lethal, 4 semi-lethal, and only one which, under special conditions, had a higher viability. Other studies both on induced and on natural mutations have given similar results. If there are among the natural mutations enough of a "progressive" kind to allow of organic evolution, and if the induced mutations do not include changes of this and allied types, but only losses, then the induced mutations as a class should be more detrimental than they have been found to be, in comparison with the natural ones.

In the case of large section changes, many, at least, of the fragments became reattached elsewhere, so that translocations and "inversions" result. When individuals containing these are bred, some offspring are produced, by recombination, which receive the displaced section in addition to two doses of the same kind of chromatin, present in its normal location. These often show phaenotypic effects of the resulting genic disproportions ("unbalance"), even when only a comparatively small piece of chromatin is involved. If, then, many of the induced "point mutations" are due to losses of small pieces of chromatin, there should also be converse cases like the above, in which the phaenotypic abnormalities were the effects of *additions* of small pieces. These additions would more usually be placed at the ends of chromosomes, as is the case with the majority of known translocations. Thus there would be an excessive number of different non-allelomorphic "mutations" found, with apparently identical loci, at the ends of the chromosomes—a state of affairs which has not been known to arise. The same kind of addition could, how-

ever, be made on different occasions at the ends of different (non-homologous) chromosomes, and sometimes at still other points ("insertion"), and so we should have the paradoxical phenomenon of allelomorphs occupying different loci, as well as the above-mentioned phenomenon of non-allelomorphs occupying identical loci.

While it is true that such additions would sometimes fail to give phenotypic effects visibly different from the normal, even when they were homozygous, yet it must be remembered that they would arise in connection with the losses of which they formed the converse. When a genically normally proportioned individual (F_1 from the treated parent) having such a displaced section (that is, "loss" plus "addition") is bred, the effect of the loss, at least, would often be detectable in those individuals of the next generation (F_2) which received this loss without receiving the displaced section, but the latter would behave as though it were a "suppressor" of this loss, lying at another locus, and thus peculiar ratios of the mutant character would be produced in this generation (F_2), the first generation in which the "mutation" would be distinguished. Such effects have not been found in the X-ray work to date, except of course in the case of the admitted translocations and inversions of large size. Many of the experiments, however, have been done in such a way that such effects would have been detected in them, had they occurred. Hence the data on these matters corroborate those previously referred to, in indicating that most of the induced "point mutations" do not consist of losses or additions of small chromosome sections.

III. A PARTIAL SEPARATION OF THE DIFFERENT GENETIC EFFECTS OF X-RAYS

That X-rays and related radiation should produce more than one kind of effect upon chromosomes and genes is not surprising; it is rather to be expected in the light of the indiscriminate metamorphosing influence which the rays have upon matter of all sorts. In the mixed medium of protoplasm, the effect of high-frequency radiation may be compared to the rampages of a bull, not so much in a china shop as in a pastry shop. Even the purely genetic effects are rather multiple than simply dual. This will be realized better when attention is called to the influence of X-rays in causing primary non-disjunction (MOHR, MAVOR, ANDERSON) and in temporarily altering the frequency of crossing over (MAVOR, MULLER), as well as in causing point mutations and losses and displacements of chromosome parts. It would obviously be far-fetched to contend that the

induced non-disjunction is brought about by essentially the same kind of interference with the genetic structure or mechanism as is the chromosome breakage; in fact, the stages in the germ cycle at which the two phenomena are most readily induced are different. Here at least, then, there are two genetic effects of irradiation that cannot be regarded as mere quantitative or spatial variants of the same genetic phenomenon.

The argument that the induced "point mutations" are only small losses and displacements because they and the large losses and displacements are produced by a common agent (X-rays) loses still more of its force in the light of some recent experiments of one of the authors, which show that changes of these two classes are not always produced with equal relative readiness by this common agent. That is, it is possible partially to separate the production of these two effects. This finding was made as a consequence of some experiments carried on during the past year (1928-1929) to study the genetic effects of irradiation upon female germinal tissue under various conditions. The point in question becomes evident when the results here obtained are compared with those from experiments in which spermatozoa were treated.

The females used for irradiation contained the dominant sex-linked gene for Bar eyes, but were otherwise normal. They were crossed in separate cultures to males containing as "markers" the recessive genes for "scute," vermilion eyes, and forked bristles, which lie scattered along the X chromosome at convenient distances. The heterozygous F_1 females were then bred in separate half-pint bottles (records of their relationships being kept) and the male offspring (F_2) of each were carefully examined in order that lethals, visible mutations, and inherited reductions of crossover frequency—the latter being indicative of displacements of chromosome sections—might be detected. In each case, except that of the two lethal point mutations marked "(?)" in the table, it was possible, by comparison of sister cultures, to make sure that these variations were newly arisen, not derived from some generation previous to the P_1 .

There were three groups of the P_1 females, distinguished by their physiological states at the time of treatment. The females of the first group (A) had been kept virgin, and in a condition of semi-starvation (by having them crowded together in a small vial, upon old, partially dried food) for a week previous to the irradiation. Those of the second group (B) had been kept virgin and well-fed. Those of the third group (C) had been allowed to mate at will, and were fed well, for the week preceding irradiation. They were all given the "t4" dose (approximately the same as the

'D5')⁴, and immediately afterwards put into fresh culture bottles, with

TABLE 1

Results of irradiation of Bar females (dose "t4") (P_1 cross: $\frac{B}{B} \varphi \times s.v.f \sigma$; F_1 cross:

$$\frac{B}{s.v.f} \varphi \times s.v.f \text{ and } B \sigma).$$

	CONDITION OF P_1 FEMALES AT TIME OF IRRADIATION	TOTAL NUMBER OF FERTILE CULTURES FROM F_1 FEMALES	NUMBER OF F_1 - F_2 CULTURES WITH MUTATIONS IN:							
			X DERIVED FROM P_1 FEMALE				X DERIVED FROM P_2 MALE			
			POINT MUTATIONS		SECTIONAL DISLOCATIONS (CROSSING OVER REDUCED)		POINT MUTATIONS		SECTIONAL DISLOCATIONS (CROSSING OVER REDUCED)	
			Lethal and semi-lethal	Visible	Lethal	Non-lethal	Lethal and semi-lethal	Visible	Lethal	Non-lethal
A	Starved, virgin	214	5(+2?)	0	0	0	0	0	0	0
B	Fed, im-pregnated	249	6	1	0	(1?)	9	0	1	1(+1?)
C	Fed, virgin	298	6	1	0	0	1	0	0	0
Total		761	16(+2?)	2	0	(1?)	10	0	1	1(+1?)

⁴ The factors of dosage for the so-called "t4" dose are as follows: filter, 1 mm aluminum; peak voltage, 50 K.V.; milliamperes, 5; distance, 16 cm; duration, 48 minutes. In some cases the milliamperage was doubled (that is, made 10) and the time cut in half, often, too, the distance was shortened and the time then reduced proportionately to the square of the distance; in all such cases the total energy is the same and the treatment is designated as "t4." Treatments in the earlier experiments (1926 and most of 1927) were, however, given with a different machine from the later ones, and we have found that the "t4" treatment, involving the above factors, on the old machine, belonging to Doctor DALTON RICHARDSON, was in reality about three times as strong as the "t4" treatments involving the same factors, given later, on the new machine of the same make (Victor, with Snook transformer) acquired by our own laboratory. In the present paper, whenever the treatment was given on the old machine, it will be so stated—for example, "t4(old machine)"—and when the machine is not designated, it may be understood that the new machine was used. "t4(old machine)," which is the "t4" of the earlier papers, must therefore be understood to be the equivalent to our present "t12."

In the experiments of one of us, a somewhat different series of time factors has been used, and the resultant dosages, all given on the new machine, have been designated in terms of "D," (PATTERSON 1929b). The dosage "D5" here referred to would be the same as "t6" (new machine), and, in general, $t1 = D1.08$, or $D1 = t0.93$.

We have found recently by means of dosimeter measurements that doses given only in terms of the above factors are far from accurate. The "t1" on the new machine may represent a dosage as high as 300 r units or as low as 150. Hereafter, where dosage is to be accurate, r units must be measured at the time.

males, and allowed to remain there for a week, laying the eggs that produced the F_1 females which were tested. The results of the tests of these F_1 females are summarized briefly in table 1. The minutae of the data from this experiment are not given here, since, as will be seen, the three groups showed no significant differences from one another, and since the chief interest of the work, from the standpoint of the present paper, attaches rather to the totals. These totals, for variations of different kinds, in the X from the treated female germ cells, are to be compared with the corresponding totals from experiments in which spermatozoa were treated.

Reference to table 1 shows that, of the 18 to 20 mutations there listed in the X from the treated female germ cells, all but one doubtful one behaved as point mutations. The exception was a case which was lost, due to sterility of the offspring, before it could be determined by subsequent breeding whether the chromosome abnormality was in the X of maternal or in that of paternal origin (the latter in this case having received the treatment also).⁵ Of the 11 or 12 mutations in the X from treated sperm, on the other hand, the number which contained distinct chromosome abnormalities was 2, or 3 if the doubtful case above alluded to is to be regarded as having been in the X of paternal origin. Only about a third as many of the sperm used had received treatment as of the eggs.

As the data relative to mutations in the X chromosomes from treated sperm are meagre in the above experiments, they may be supplemented by citation of an experiment in which the P_1 cross was the reciprocal of the P_1 cross in the above experiment. Here Bar males were treated, and then crossed to untreated " $C_1 B$ " females. The dosage ($t13+$) was, however, much higher than in the other experiment. Here, too, there were physiological differences between different groups of the treated flies,—in this case the difference was in temperature, one group being kept warm, the other cold, while treated—but as the two groups gave essentially similar results, the data have been combined in table 2. It will be seen from this table that the total number of sectional changes is comparable with that of the point mutations, and that if anything like this ratio of the two classes had existed in the treated female cells of the experiment reported in table 1, the results obtained would surely have been different, despite the small total numbers of mutations there dealt with.

⁵ Chromosome abnormalities do sometimes occur in the chromosomes of irradiated females, however. In group A, for example, an F_1 fly was found which proved to have a duplication on one of its third chromosomes—a piece from the middle of the genetic map of the left arm, not including the end, having become attached to the right end of an otherwise normal third chromo-

Further corroboration of the point in question may be obtained from the data of the original X-ray experiment, obtained in 1926 (MULLER 1927, 1928b), although these would, by themselves, hardly have been extensive enough, on the female side, to be conclusive. The figures pertinent

TABLE 2

Results of irradiation of *Bar* males (dose "113"). P_1 cross: $\frac{s_{\text{vfb}}}{\text{"CIB"}} \text{♀} \times B \text{♂}$; F_1 cross: $\frac{s_{\text{vfb}}}{B} \text{♀} \times s_{\text{vfb}} \text{♂}$.

	TEMPERATURE OF P ₁ MALES AT TIME OF IRRADIATION	TOTAL NUMBER OF FERTILE CULT- URES FROM F ₁ FEMALES	NUMBER OF F ₁ -F ₂ CULTURES WITH MUTATIONS IN:						
			X DERIVED FROM P ₁ FEMALE	X DERIVED FROM P ₁ MALE					
				POINT MUTATIONS			SECTIONAL DISLOCATIONS		
				Lethal	Semi-lethal	Visible	Lethal	Visible	Invisible
A	6° ± 2°C	36	0	5	1 (visible)	3	5	1	4
B	34° ± 1°C	62	0	12	2 (visible)	3	6		5
Total		98	0	17	3 (visible)	6	11	1	9

to the matter at issue are given in table 3. From the treated male cells, there is approximately the same proportion of sectional to point

TABLE 3

Results of treatments given October, 1926.

X-RAY DOSE (OLD MACHINE)	SEX IN WHICH CHROMOSOME WAS TREATED	TOTAL NUMBER OF FERTILE F ₁ -F ₂ CULTURES	NUMBER OF F ₁ -F ₂ CULTURES WITH MUTATIONS IN X DERIVED FROM TREATED PARENT.						
			POINT MUTATIONS			SECTIONAL DISLOCATIONS			
			Lethal	Semi-lethal	Visible	Lethal	Semi-lethal	Visible	Invisible
t2	♀	216	12	4	1	1	0	0	?
t2	♂	65	6	1	1	4	0	0	?
t3	♂	72	5	1	3	3	1	1	?
t4	♂	405	38	7	12	16	2	1	5(+?)
Sum ♂	♂	542	49	9	16	23	3	2	5(+?)

changes as in table 2, when allowance is made for the fact that the "invisible" sectional changes were not specially looked for, and that hence probably only a small fraction of those occurring were detected. But, again, the chromosomes from treated females show a far smaller number of

chromosome abnormalities, in proportion to the point mutations, there being only 1 to 17, whereas those from the treated males given the same dose show 4 to 8 and those from all treated males combined show 33 sectional to 75 point changes.

Taking these three experiments together, then, it seems safe to conclude that under certain conditions not nearly so many sectional changes can be obtained by treatment with X-rays, in proportion to the point mutations simultaneously obtained, as under other conditions. The decisive conditions seem, in the present experiments, to be somehow connected with the sex of the cells undergoing treatment, though whether the connection with sex is causal or accidental is as yet somewhat uncertain. It is possible that the different average dosages given the males and females may also have played a part. But, be the basic determining conditions what they may, the important point here is that, by their means, one of these two processes may be influenced largely separately from the other. Accordingly, there must be some real difference between the mechanisms whereby the sectional and point changes occur, and, though there may also be some feature or features common to the two mechanisms, as suggested by the fact that both can be initiated by X-rays, nevertheless there remains no reason to make the specific assumption that the difference between them is purely quantitative, rather than of some other nature.

It is easy to conceive of ways in which the two processes might be related so that they would be affected in the manner found. For example, the breakage of a chromosome, as well as the change in composition of a single gene, unaccompanied by breakage, might be due in the first place to the breaking of a single chemical bond, by the displacement of an electron, followed by rearrangement of the interatomic associations. When the rearrangement occurred within a gene, in such a way as to leave it still a gene (that is, capable of multiplication) in spite of its change, and still connected with its neighbor genes, a "point mutation" would have occurred. But when the rearrangement happened to be such as to destroy the gene (that is, to leave it no longer with the power of multiplication), or to break its connections with its neighbor genes, then it might be supposed that a break in the chromosome would result, though perhaps not until after the chromonema-envelope previously existing had become used up or replaced by a new one. In some cases, the broken chromosome-ends would probably join with each other again; in some other cases, we know that they become attached to other chromosomes, or at other places on the same chromosome. Now this latter phenomenon, the process of attach-

ment, might well be subject to different influences than the original process of inter-atomic rearrangement above postulated was subject to. It might, for instance, be much more likely to occur when the chromosomes were in a particular physiological condition, or when they were packed together tightly morphologically, as they are in the sperm-head. Under such circumstances, then, displacements of chromosome sections would be especially likely to occur, though the number of "point mutations" need not be correspondingly more frequent. We have sketched here, however, but one out of numerous possible interpretations. It would not, at the present stage of our knowledge, be profitable to speculate upon the matter in more detail, particularly since certain further tests bearing upon it can be made.

IV. THE PRODUCTION OF MULTIPLE ALLELOMORPHS

In the eyes of many geneticists, the most convincing line of evidence that has been brought against the "presence and absence" theory in general has been the phenomenon of multiple allelomorphism, with its attendant features. There is now evidence indicating that this same phenomenon, in all its details, can be induced by X-rays. If so, then the same series of arguments as have been found applicable in the case of this general question (MORGAN, STURTEVANT, MULLER and BRIDGES 1915, 1923, MULLER 1919, 1920) now apply similarly against the idea of "presence and absence," or "mutation by loss alone," in the more specific case of the changes induced by X-rays. The experimental evidence concerning this matter will therefore be in place here.

The locus in *Drosophila* at which the greatest total number of "spontaneous" mutations, and also the greatest number of different looking "spontaneous" mutations, have been detected, is that of white eye (MULLER 1920). Of the dozen or more different mutant allelomorphs of spontaneous origin known at this locus, white has been found by far the oftenest (over a dozen times, possibly two dozen), eosin several times, and most of the others just once in all the *Drosophila* work to date. In the X-ray experiments, likewise, it has been this locus in which the most mutations have been discovered, and it is likewise found that most of the mutations induced at this locus have given rise to the allelomorph white. The latter has been induced by irradiation as a mutation in a germ cell on more than a dozen different occasions in our laboratory. From the combined results (see table 4), it may be calculated that it arises in something like 1 in 1000 X-chromosomes treated with our heavy "t12" (or "D13") dose, in the mature spermatozoa. And, in addition to white, several other mutant

allelomorphs at this locus have been found in our laboratory, after raying (MULLER 1928 a, c).

One of these allelomorphs is eosin, found by a graduate student, Miss CAMPBELL, in May, 1927, in an experiment directed by one of the authors to test the frequency of production of translocations. The eosin arose from the normal allelomorph, in a chromosome carrying also the mutant genes for scute, vermilion, and forked. A male (P_1) bearing these genes had been heavily X-rayed (dosage, "t4, old machine") and mated to a female having only normal genes in the X chromosome. In the male progeny (F_2) of one of the F_1 females, there was the expected count so far as the characters that were supposed to have entered the cross were concerned, but the individuals carrying scute and vermilion were of a lighter eye color. By subsequent breeding, the gene responsible for this effect was separated from scute and vermilion, and was found to produce the peculiar color of eosin, in the sexually dimorphic fashion characteristic of the latter. Its locus also was determined to be at about 2.0, with reference to scute, and when crossed with white it gave a light eosin color, as does the familiar eosin. As there had been no eosin stock in the university except one which carried no other mutant genes, it is unreasonable to suppose that eosin could have crept into this particular combination with the expected genes, scute, vermilion, and forked, by contamination. The evidence is therefore complete that in this case eosin arose from the normal allelomorph by mutation, after irradiation of the sperm.

A second allelomorph of white, found by one of the authors in the fall of 1928, is to all appearances identical with the known allelomorph, "apricot." It arose in the experiment summarized in table 2, in a culture descended from the flies that had been kept at the colder temperature (6°C) during treatment. The F_1 female among whose progeny it was found had received from her mother an unirradiated X chromosome with the genes, scute, vermilion, forked, bobbed, and from her father, a radiated X, containing Bar, in which a lethal inversion had just arisen somewhere in the right region, and the gene for apricot in the left. The male progeny, therefore, included only one non-crossover class ($s_c \ v \ f \ b_k$), the other noncrossover class (B) dying. The former non-crossovers had the expected characteristics. There was, in addition to these non-crossovers, only one crossover male, due to the reduction of crossing over caused by the inversion, and to the fact that crossover males receiving the left end of the unirradiated X and the right end of the radiated X died. The crossover male which appeared was of the contrary class to this; it carried the normal allelomorph of scute (therefore the radiated left end) and the mutant genes

for vermilion, forked, and bobbed (the unirradiated right end), but it had a light lemon-like eye color, indicative of the new mutant gene (apricot) in the left-hand portion of it, derived from the radiated X. As a result of crosses between this male and normal females, numerous crossover males were obtained in F_2 which carried the new mutant without the genes $v f b$, and a pure stock was derived from these, in which it was evident that both males and females had the eye-color characteristic of the known apricot. Crosses with white resulted in an eye color of intermediate shade in the daughters; this showed that the new mutant was really an allelomorph at the locus in question.

A probable third allelomorph was obtained by PATTERSON in 1929, from a cross between a radiated (D10) eosin singed male and a yellow female with attached X's and a Y chromosome. The sons from such a cross, receiving their father's X and mother's Y, would ordinarily be eosin singed, like their father. The great majority were, but one appeared very much lighter in color than the rest. Through crossing, the new gene has been separated from singed, and a pure stock of it has been obtained, in which it is evident that the females are somewhat darker than the males, as is true of eosin, from which the new gene was derived, and of another known allelomorph called ivory; the flies are consistently lighter than eosins, however, and probably lighter than ivory. The fact that heterozygotes, carrying one dose of eosin and one of the new mutant, are of intermediate color indicates that the case is one of allelomorphism rather than of modifying genes.

A probable fourth allelomorph arising in somatic tissue as a result of raying the allelomorph apricot in an embryonic stage will be referred to subsequently, in connection with the account of reverse mutations.

Nine cases of mottled eyes have also been found by the authors (MULLER 1928 a c, 1930). These are recessive to red and give intermediates when crossed with white and the other mutant allelomorphs of this locus. They vary through all the colors known in this series, and more. However, the mottleds, unlike the mutants of uniform color above described, do not behave as simple point mutations, but, without exception, involve breakages and reattachments of chromosome parts; accordingly they do not furnish material for illustrating the principles here under discussion.

Another locus in which several "point mutations," including different allelomorphs, have been induced by X-rays is that of forked bristles (f). The normal allelomorph of forked has mutated on three different occasions

in experiments of one of us involving irradiation of mature spermatozoa, not designed specifically for studies of the mutability of this particular locus. In the first case (spring of 1927), males having the gene for small eye, but otherwise normal, were given the t4 (old machine) dose and mated to females carrying in one chromosome the " C_1B " combination and in the other $s_e v f b_b$. In a section of this experiment in which the males were held for six days after treatment, before mating, 243 F_1 females inheriting the $s_e v f b_b$ chromosome from the mother were produced. Among the latter, one was a typical forked which, on breeding, proved to be homozygous for the same, having a new gene for forked in the irradiated chromosome, with the gene for small eye, and no evidence of any X-chromosome abnormality. Pure stock of the new forked had good viability and fertility, and exhibited the character in typical fashion.

In another experiment (fall, 1928) males containing the gene for bobbed bristles (b_b) and an inversion designated as " $\delta 49$ " were given a t16 dose and crossed to yellow attached-X females. Among the 615 male offspring, one was a typical forked, which transmitted its mutant character to its offspring as a sex-linked recessive. Crosses with the old forked resulted in forked daughters; this showed the new gene to be allelomorphous to the old.

A third mutation in this locus occurred in the experiment shown in table 2 in the "warm" series. Among the 120 F_1 females tested in this series, one which had received " C_1B " from its mother and an irradiated Bar-containing X-chromosome from its father yielded sons all of which were both forked and Bar. Further study proved the new forked to be in the same locus as the old, but it was noticeable that in the new stock the forked character was not nearly as well marked as in the typical stock of the old forked. As it is unlikely that a modifier had happened to arise in just the same chromosome as the gene for forked itself, it is probable that there was here a different, and "weaker," allelomorph, " f^w ," such as has been found in some of the previous *Drosophila* work on material not artificially irradiated. In one of the following sections of the present paper, dealing with reverse mutations, another case is recorded of the origination of a "weakly forked" allelomorph (considerably weaker than the foregoing), and likewise of other mutations at this locus, after irradiation, in experiments especially intended for the study of changes at this locus.

The occurrence of two mutations from non-scute to typical scute, subsequent to irradiation, will also be described in a following section. In addition to these mutations to scute, there has been one giving rise to

a distinctly different mutant allelomorph (shown in figure 1). This arose in an experiment in which wild-type adult males were given a t13 (D14) dose, and then mated to females having in one X chromosome the s_c C_1B s_m v t_n complex and in the other s_c v f b_b . Four hundred thirty two fertile F_1 females that had received C_1B were bred in separate vials, the progeny (F_2) being examined through the walls of the vials under the low power of the binocular. In this method of examination, devised by C. P. OLIVER, the vial is held under the binocular in a horizontal position with the stopper towards the light; the flies then congregate against the upper wall of the vial, next to the stopper, and any conspicuous visible mutations present in the males as a group can be readily detected (as well as the absence of males, indicating a lethal). Among the 432 cultures of the

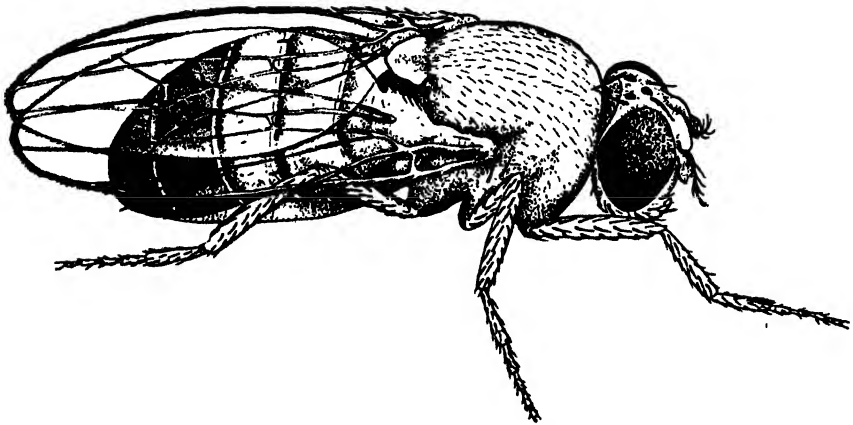


FIGURE 1 —The mutant "scutex" produced by irradiation.

type stated, one was found in which there were only a few males, and these were all of peculiar appearance, while all the females appeared to be scute. On anaesthetization and examination under higher power, it was found that the males were of a very extreme scute type, completely devoid of bristles on the dorsal surface of the thorax and scutellum. The body color also appeared to be somewhat lighter, and the wings more transparent looking than in the normal, so that the whole fly had a delicate, flimsy appearance.

The females in the above cultures, on being bred to scute (attempts to breed or keep alive the males with the new character proving unsuccessful) gave rise to offspring all of which showed either scute or the new mutant. Hence either a new allelomorph of scute had arisen, or scute and an intensifier had arisen simultaneously. The latter inherently improbable

assumption was made still less tenable by the crossover ratios, which showed the effect to be located at the left end of the X chromosome, like scute itself. The new allelomorph, which may be designated as "scutex" (s_c^x), was found to act as a semi-lethal, inasmuch as, in most cultures, fewer than a tenth of the flies expected to show it actually hatch. In combination with the old scute, the latter dominates, but *not completely*: that is, there is on the average somewhat more reduction in the number of thoracic bristles in the heterozygous s_c^x/s_c females than in the homozygous s_c/s_c . As shown in the figure, the artist found postvertical and subhumeral bristles present, although these are absent on scute flies, which have a greater tendency to produce bristles in the other positions. We have not been able to check up on this point in pure scutex, since the latter has recently been too inviable, but in compounds with scute we find the postverticals and subhumeral to be absent. If they were really present oftener in scutex than in scute it would be evident that these characters were not different merely in a quantitative way.⁶ They would be related non-quantitatively in a somewhat similar manner to that first found by MULLER to hold in the case of the allelomorphs of the truncate series (vortex, oblique, dumpy, lopped, thoraxate, truncate; MULLER 1919, 1923).

Perusal of the above three cases of multiple allelomorphism, following upon irradiation, will bear out the contention that they exhibit all those characteristics which, in the case of the natural multiple allelomorphs, have been taken as evidence against the idea of mutations in general consisting of losses. For one thing (1), the allelomorphs are surprisingly frequent if they represent only the last term—the most extreme possible cases—of close linkage between genes at essentially different loci. Cases of merely very close linkage, though occasionally found, are on such a view relatively much less frequent than they should be, and the advocate of "presence and absence" is thereby forced to some such subsidiary hypothesis as the existence of intra-chromosomal groupings or "nests" of genes.

Secondly (2), the different mutant allelomorphs in any one of the three series affect the same general character (eye color, bristle conformation, or bristle distribution, as the case may be). There is no known or apparent

⁶ Since the above finding was made on scutex, the authors' attention has been called to a certainly non-quantitative, and much more extensive series of allelomorphs of scute, arising after irradiation, in experiments of SEREBROVSKY, DUBININ and their colleagues (SEREBROVSKY, DUBININ, AGOL, SLEPKOV AND ALTSHULER 1928, DUBININ 1929). The reports on these, however, were not, at date of writing, available to us.

reason why this should be true if the different allelomorphs in a series are simply losses of different completely linked (neighboring) genes, but it is readily understandable if they are different changes in one gene, that has become specialized to react chiefly, so far as visible characters are concerned, in the development of this particular character.

Thirdly (3), whenever individuals with different mutant allelomorphs are crossed, instead of the normal type becoming reconstructed phenotypically in F_1 , as it is when individuals with recessive non-allelomorphic genes are crossed, the F_1 shows the characteristics common to the mutants that were crossed, and, in any respect in which they are different, and both abnormal, it is no nearer to the normal than the allelomorph that is more normal in this respect. To explain this non-appearance of the normal in F_1 on the "presence and absence" hypothesis (of different completely linked genes that become lost), requires the special assumption of the linked genes observing a precedence in regard to the order in which they are lost. On the presence and absence view, the less extreme allelomorph is considered to lose a gene, which may be designated as "*A*"; then the more extreme one, in order, on crossing with the other, to yield a hybrid showing at least as much variation from normal as shown by the less extreme allelomorph, must likewise lack *A*, but it must lack something else, "*B*," in addition, which distinguishes it from the less extreme allelomorph. Thus, *A* can be lost by itself, but *B* is never lost unless *A*, which has the precedence, is lost with it. This assumption must be carried to considerable lengths in the case of the series of X-ray allelomorphs of white, where apricot must be considered to have lost *A*, eosin to have lost *B* and *A*, the lighter mutant from eosin above mentioned to have lost *C*, *B*, and *A*, and white to have lost *D*, *C*, *B*, and *A* simultaneously (or many more, if the allelomorphs of spontaneous origin also are taken into account). On the other hand, the assumption of no such hierarchy of genes is necessary on the view that the allelomorphs are merely different changes in one gene, which alter its reactivity to various extents, and in various ways. On this view the usual lack of reconstitution of the normal type, in crosses between two mutant allelomorphs, ceases to be paradoxical.

To meet such objections, the believer in "presence and absence" would now have to resort to some additional postulates, such as that the completely linked genes formed a cluster of identical "elements" or parts, any one or more of which could be lost, and the number of which determined the degree of expression of the character. Among the numerous difficulties that would arise for such a view only two need be mentioned here. One is the difficulty that would be met with in accounting for the

consistent dominance of the normal allelomorph in these cases. (Apparent normals should arise, which did not have a sufficient number of elements to dominate.) A far more serious obstacle is encountered in the fact that, in the case of the white and probably the scute series, the members do not form a purely quantitative series at all. Apricot, unlike eosin and the lighter variant from eosin, is alike in color in male and female; scute, though having far less extreme bristle reduction than scutex in most respects, nevertheless probably lacks certain bristles more often appearing in the latter. If there has been loss, then, the parts lost have been somehow different from one another, and yet these various parts were obviously related to one another in their functioning, in a much more intimate way than that in which genes in different loci are ordinarily related. The readiest method of explaining their peculiarly intimate relationships is to assume that they were chemically united. But we cannot tear off a piece of a molecule without healing the broken bond, either by a rearrangement of the remainder, or, as is far commoner, by the addition (substitution) of something else, large or small, in the place made vacant. In either case, the idea of a pure loss becomes vitiated. In truth, there is no theoretical reason left for assuming that a loss would be the exclusive method of change of the gene.

In connection with these allelomorphic series, it is pertinent to put the question, "Were the different allelomorphs all really produced by the irradiation, or were only the commoner (usually the most extreme) allelomorphs so produced, and the others of 'spontaneous' origin in each case?" While this question cannot be given an absolute answer, it can be met in terms of strong probabilities. Thus, in the case of the white-locus series, in all the experiments on treated mature spermatozoa combined there was a total of somewhat less⁷ than 22,366 flies that would have served for the detection of white or one of its allelomorphs (table 4). Seven thousand two hundred fifteen of these were F_1 males derived from treated males crossed by attached-X females, and 15,151 were F_1 females (derived from mothers with separate X's) which were bred in individual cultures, and the male progeny of which were examined. In this total there were 10 or 11 that carried white, and 1 that carried an allelomorph (apricot) as a heritable germinal mutation, and two males that carried white "fractionally" in some of the somatic and not in the germinal tissue.

The "fractional" white mutations that were confined to the soma should probably be counted at a value of at least $\frac{1}{2}$ each. It is likely that there were parallel cases in which white occurred in the germinal tissue but in

⁷ Lethal-bearing flies are to be subtracted from the total.

which the male was not bred because there were no somatic indications of the mutation; the latter cases would, however, be less frequent than the former, since at least one of the two eyes is usually derived from the same nucleus of the two-cell stage as is the germinal tissue. The use of the factor $\frac{1}{2}$ will therefore serve to keep our results on the side of caution. Taking 13, accordingly, as the total number of whites, we find that white arose about once in 1800 sperm cells and a different allelomorph about once in twenty-three thousand sperm cells (in round numbers), in this work. In all the previous *Drosophila* work on non-radiated material, up to 1925, it has been estimated that upwards of twenty million flies have been examined (MORGAN, BRIDGES and STURTEVANT 1925). Nearly half of these must have been males in which white or one of its allelomorphs would have been pretty sure to be detected (and something like one one-hundredth must have been females subjected to the progeny test and similarly serviceable). Among the 10,000,000 thus available, white has been observed to originate only about two dozen times, at the most, and all other allelomorphs of white, combined, not much over one and a half dozen times. This makes a frequency for white of about one in four hundred thousand times, and, for the other allelomorphs, of about one in six hundred thousand times. In the total of less than 23,000 in the series of radiation experiments above referred to, there was, therefore, about 1 chance in 17 that white should have appeared at all, and 1 in approximately (17)¹² that it should have appeared as often as 13 times, unless there had been some peculiarity in the conditions of the experiment responsible for producing it. In like manner, the chances that one of the other allelomorphs, such as apricot, should have appeared once, would have been only 1 in 26 if the material had not been somehow made especially mutable.

It can readily be calculated from the above figures that, in all, the frequency of appearance of mutations at the white locus in the irradiated sperm was of the order of magnitude of 200 times the corresponding frequency in non-radiated material. If, now, we weight the flies in all the experiments according to the dosage of radiation used upon the sperm,—which is legitimate, in view of the direct proportionality between dosage and mutation rate found by HANSON and HEYS and by OLIVER—we find that there would have been 1 detectable mutation of the normal allelomorph of white to some mutant allelomorph or other among about 1,000 sperm treated with the heavy “t12” (or D13, or “t4, old machine”) dose. The items in this calculation are shown in table 4. This result is to be compared with 1 in about 350,000 in the non-radiated material. It will be seen that the former frequency is about 350 times the latter—a

TABLE 4

Summary of results with regard to loci of s_e , w , and f , from treated sperm (except for experiments recorded in tables 9 and following).

1.	2.	3.	4.	5a	5b	6.	7.	8.	9.	10.
DESIGNATION	WORKER	DATE OF EXPERIMENT	METHOD OF DETECTION*	NATURE OF CROSS† (TREATED GENES IN HEAVY TYPES)		NO. OF FLIES TESTED OR INSPECTED	DOSE (IN "U")	FACTOR OF X-RAY MACHINE (OLD=3, NEW=1)	"UNITS TESTED" (PRODUCT OF 6×7×8)	MUTANTS AT ABOVE LOCI
				♀ PARENTS	♂ PARENTS					
"first"	M	fall, 1926	F_2	$(F_1) \frac{b_b}{s_e f}$	$(F_1) \frac{s_e f}{Y}$	25 65 72 405	1 2 3 4	3 3 3 3	75 390 648 4,860	$1w$
"aged sperm"	M	spring, 1927	"	$(F_1) \frac{s_v}{s_e C_l B_v}$	$(F_1) \frac{s_e f}{Y}$	619 567	4 2	3 3	7,428 3,402	$1f$
"fractional"	M	spring, 1927	$F_1 \sigma^7$	$(P_1) \frac{y y}{Y}$	$(P_1) \frac{b_b}{Y}$	1,150 1,490	4 2	3 3	13,800 7,940	$\frac{1}{2}w$ $1w$
"stability"	M	fall, 1927	"	$(P_1) \frac{y y}{Y}$	$(P_1) \frac{C y}{Y} + \frac{S_v}{Y}$	2,080	4	3	24,960	$\frac{1}{2}w$ $\frac{1}{2}w$
"849 loci"	M	fall, 1928	"	$(P_1) \frac{y y}{Y}$	$(P_1) \frac{849}{Y}$	615	16	1	9,840	$1f$
"849 loci" No. 2	O	fall, 1928	$F_1 \sigma^7$	$(P_1) \frac{y y}{Y}$	$(P_1) \frac{849}{Y}$	\int 376 1,504	16 1	1 1	6,016 1,504	$1w$ (died)

TABLE 4 (continued)

"translocation"	M	winter, 1927- spring, 1929	F ₂	back cross for F ₁ mar- kers in II and III		282	9.3 [±] · · ·	1 [±]	2,616	10 [±] ; j/f [±] (from (from cold) warm)
"temperature"	M	fall, 1928	"	$(F_1) \frac{B}{s_c f b_b}$	$(F_1) \frac{s_c f b_b}{Y}$	187	12	1	2,244	
"sex"	M	spring, 1929	"	$(F_1) \frac{b_b}{s_c C_1 B_v}$	$(F_1) \frac{s_c f b_b}{Y}$	402	8	1	3,216	
"inversion"	M	spring, 1929	"	$(F_1) \frac{849B +}{s_c C_1 B_v}$	$(F_1) \frac{s_c f b_b}{Y}$	856	14	1	11,984	
"aged sperm"	M	spring, 1927	F ₁ ♀	(for loci of s _c and f only) $(P_1) \frac{s_c f b_b}{s_c C_1 B_v} (P_1) \frac{s_y}{Y}$		619 567	4 2	3 3	7,428 3,402	
"metabolism"	M	winter, 1928- 1929	F ₂	(for locus of z only) $(F_1) \frac{s_c f b_b}{B} (F_1) \frac{s_c f b_b}{Y}$ and $\frac{B}{Y}$		260	4	1	1,040	

TABLE 4 (continued)

DESIGNATION	2.	3.	4.	5a	5b	6.	7.	8.	9.	10
	WORKER	DATE OF EXPERIMENT	METHOD OF DETECTION*	NATURE OF CROSS† (TREATED GENES IN HEAVY TYPES)		NO. OF FLIES TESTED OR INSPECTED	DOSAGE (IN "Y")	FACTOR OF X-RAY MACHINE (OLD = 3; NEW = 1)	"UNITS TESTED" (PRODUCT OF 6 X 7 X 8)	MUTANTS AT ABOVE LOCUS
				♀ PARENTS	♂ PARENTS					
"dosage"	O	winter, 1928-1929	"	(for locus of <i>w</i> only) $(F_1) \frac{+}{s_c C_1 B v}$		8,444	3 ^x	1	24,958	5 ^w
"aging of males"	H	winter, 1928-1929	"	(for locus of <i>w</i> only) $(F_1) \frac{b_b^\dagger}{s_c C_1 B v} \frac{s_c y b_b}{(F_1) \frac{s_c y b_b}{Y}}$		2,967	8	1	23,736	3 ^w
Total for <i>w</i>	M+O +H					22,366	(6.6 ^x)	(. . . 1 ^x)	150,657	13 ^w +1 ^{wss}
Total for <i>s_c</i> and <i>f</i>	M+O					11,881	(9.0 ^x)	(. . . 1 ^x)	111,753	2 ^f +1 ^w +0 ^{s_c}

* F₁♂ = inspection of F₁ males; F₁♀ = inspection of F₁ females; F₂ = testing of F₁ females by inspection of F₂ males.† Where detection is by inspection of F₁, composition of P₁ is given; where F₁ are tested, their composition is given. Symbols are as follows:
Y = Y chromosome.

yy = double X chromosome, homozygous for yellow body color.

+ = normal chromosome.

† From early broods of P₁ males.

x = average

Controls to above experiments have totalled over 10,000 flies tested, without a single *w*, *s_c*, or *f* having arisen.

factor even greater than that expressing the increase in the frequency of lethals at the same dosage. (There is, however, more chance for errors in detection to affect the figures for white than those for lethals in untreated material). There can, therefore, be little doubt that the radiation was responsible for these mutations of the white locus.

Similar calculations can be made with respect to the mutations in the loci of forked and of scute. The results here will not be as accurate as in the case of the white locus because of the fact that these characters, being somewhat less conspicuous than those of the white series, are more apt to have been overlooked, especially in the non-radiated material. Nevertheless, the chances of detection of both forked and scute are distinctly good; once an investigator has worked with them, he is not likely to overlook them. In all our work on the progeny of radiated sperm combined, including that reported in the subsequent sections of this paper, there have been 29,402 F_1 flies in which a mutant gene for scute (received from treated sperm) would probably have been detected, and 5961 F_1 females in which, by progeny tests, scute would probably have been found. In the summarized *Drosophila* work to 1925, scute was reported 4 times among the 10,000,000 males examined, and scutex not at all, among some 200,000 females whose male progeny were examined. These results would have given a chance of only 1 in about 71 of finding one scute mutation, or 1 in about 5000 of finding two of them, in a series of experiments of the magnitude of the above irradiation experiments, while the chance of finding scutex in these experiments would be less than 1 in 33, and would really be too small to be reckonable from the data. In all, the rate of mutation to scute found in sperm given a t12 (or t4, old machine) dose can be figured to have been about 250 times the rate in untreated cells.

The case of forked will be considered in greater detail later, when additional data more specifically concerned with mutations at this locus will be presented. Aside from these subsequent data, however, the previous work on the progeny of irradiated sperm have considerable significance. There were in this work in the neighborhood of 11,881 flies from treated sperm, in which a gene for forked would have been detectable. (The dosages were such that these would have been equivalent to 8,911 at the "t12" or "D13" dose.) It was among these that the two mutations to forked and one to "weakly forked" were found. Now, in the summarized *Drosophila* work, there were approximately 9 mutations to forked (or to some one of the 4 known kinds of forked allelomorphs) observed among the approximately ten million flies available for such a discovery; this makes the chance of finding one in a series of experiments of the total

magnitude of the above irradiation experiments only 1 in 90, and the chance of finding two, one in 8,100. While the weaker allelomorphs are much more likely to be missed, still it can be seen that the odds must be greatly against finding one of these in our 11,881 flies either, unless some special influence were producing them. In all, when the figures are corrected to appear as of the t12 dose, the mutation frequency to some allelomorph of forked found in the treated is over 300 times that found in the untreated individuals.

It is of course realized that in the case of this as of the other loci (white and scute) the mutations detected and reported in the untreated material represent only a fraction of those which occurred, since a special attempt was not made in most experiments to find the mutations in question, and since, even when they did occur, they were often ignored on account of the possibility of their having resulted from contamination. This probably accounts for the observed frequencies for the t12 dose being several hundred instead of about one hundred times those reported in the summarized untreated material (in view of the fact that the lethal frequency is raised only about one hundred- to one hundred fiftyfold). Nevertheless the reported frequencies of visibles in the untreated material are doubtless of the right order of magnitude at any rate, and so long as this is true, the conclusions reached from the above calculations would still hold, so high are the probabilities there arrived at.

To sum up, then, if we take all the three loci into consideration at once, it is quite evident that irradiation is really the agent which has brought about the production of the multiple allelomorphs, and, since these induced multiple allelomorphs display the same series of characteristics (with regard to phaenotypic expression, dominance relations, etc.) as do the multiple allelomorphs of spontaneous origin, all the arguments against "presence and absence" and "mutation by loss" which can be based upon the induced allelomorphs must have the same validity as they have admittedly had in the past in the general theory of heredity and variation, when they were based upon spontaneous multiple allelomorphs. It is likewise apparent that the notion of "addition" of genetic material (supposing small pieces to have become torn out of other regions of the chromatin and attached at the loci in question) is even less capable of explaining the peculiarities of the results that have been discussed above, than is the notion of mutations by loss alone. Such small-scale displacement, supposing it were possible, could not help but result in a heterogeneous collection of dissimilar mutations at a given locus showing no characteristics of multiple allelomorphs except the inability to recombine by crossing over.

V. AN INDUCED MUTATION VISIBLY DIFFERENT FROM A
KNOWN LOSS OF THE SAME LOCUS

In October, 1927, 84 fertile males carrying a normal X chromosome (and a mutant combination including Star eye in one of their second chromo-



FIGURE 2.—Dominant "eyeless" (in fourth chromosome) produced by irradiation. Below is given pair of legs with sex combs of normal size, for comparison with the enlarged ones of the eyeless fly.

somes) were given the t4 dose (old machine) and, after being kept in isolation for 16 days, mated in individual cultures to yellow attached-X females heterozygous for Curly wing. After 4 days, the parents were transferred to a second set of culture vessels (called "brood 2") and, after another 7 days,

to a third set ("brood 3"). Altogether, 2,080 F_1 males were carefully examined for mutations, and 437 F_1 females (namely, all those of brood 1). This count of males is shown on row 4, table 4. In the second brood, among the 18 F_1 males appearing in the last culture (No. 84), there was one non-Star, non-Curly male which had one eye misshapen as if furrowed, and the other eye normal. The 11 males in brood 1 from the same parent were all normal. When the aberrant male was bred back to yellow attached-X females, it was found that the variation was transmitted as an autosomal dominant, to the daughters as well as to the sons, appearing in approximately half the members of both sexes. It usually affected both eyes rather strongly, causing a change in size and shape rather similar to that seen in the dominant second chromosome mutants "Lobe" and "Lobe," in the dominant third chromosome mutant Deformed, and in the recessive fourth chromosome mutant "eyeless." Besides the eye abnormality, it produced, in the male, a hypertrophy of the sex combs, the latter being about doubled in size. Examples of the variation are shown in figure 2. Sometimes even more abnormal types are produced in this stock, the head becoming split or otherwise malformed, and developing peculiar protuberances and multiple antennae as is sometimes the case with the recessive eyeless.

Backcrosses of the males inheriting the abnormal eyes from one parent, and black body color (in chromosome II) from the other, to black females, then showed that these two genes underwent independent segregation, and that therefore the new mutant could not be in the second chromosome. Similar crosses were thereupon made involving the new mutant and peach eye, which is in chromosome III, and it was found that the mutant was likewise independent of this chromosome in its inheritance. Accordingly, it was crossed to bent wings, a recessive lying in the tiny chromosome of the fourth pair, and the F_1 females that showed the eye abnormality were backcrossed to bent males. The count of 2202 flies consisted of two major classes: bent, normal eyes, and non-bent, abnormal eyes. There was also a considerable number (219) of apparent normals, which were to be expected owing to the overlapping of the normal type by both the bent and the abnormal eye classes; a number of these normals were tested and all these fell genotypically into one or the other of the two major categories above named. On the other hand, there were no recombinations showing both the bent wings and the abnormal eyes. It was therefore clear that this gene was in the fourth chromosome, and that its locus showed complete or nearly complete linkage with that of bent (no bent-eyeless crossover combinations to 1248 bent non-eyeless and 735 non-bent eyeless).

As it was now highly probable that the new gene was a dominant allelomorph of the known recessive "eyeless," it was crossed to the latter. The F_1 flies containing the two genes in combination proved to have, on the average, a greater reduction in the size of the eye than was characteristic of either the homozygous recessive eyeless or the heterozygous new mutant, although they were seldom completely eyeless. It will then be assumed that the two genes are allelomorphic, and the new mutant will be designated as "Dominant eyeless" (e_v^D).

As all Dominant eyeless individuals tested proved to be heterozygous, even though both parents had carried the gene in question, it appeared likely that it acted as a lethal when in the homozygous conditions. Crosses were made between it and the dominant minute bristle (M_{IV}), located in the same chromosome, which also is lethal when homozygous. The F_1 flies having the heterozygous combination e_v^D/M_{IV} were next crossed to each other. This resulted in F_2 all resembling their F_1 parents. In other words, a balanced lethal stock had been established, and there could be no doubt that homozygous e_v^D was lethal. The combination of the two characters results in individuals that tend to be somewhat abnormal in bodily shape (broad and squat) but with no evidence of the rotated-abdomen condition which M_{IV} produces when combined with the recessive mutant of that name with which it is allelomorphic. The $e_v^D-M_{IV}$ combination flies have a low fertility, but, once numerous individuals are obtained, the stock can, with care, be perpetuated indefinitely.

Crosses of e_v^D to bent and shaven show that it is allelomorphic to neither of the latter. Thus, of the four loci known in the tiny fourth chromosome—namely, that of bent, of shaven, of the allelomorphs M_{IV} and rotated abdomen (TCHETVERIKOFF), and of eyeless— e_v^D shows allelomorphism to eyeless only. Hence it is unlikely that it involves a "deficiency" of a chromosome region, or any disturbance other than ordinary "point mutation."

A digression may be made at this point to call attention to the bearing of the above results on some previously published genetic maps of the fourth chromosome. In these (for example, in MORGAN'S *Theory of the Gene*, 1928, p. 23) the known loci of chromosome IV are shown separated by distinct intervals, covering in all nearly one unit, and reflecting the idea that several tenths of a percent of crossing over occurs between the loci. Examination of the experiments on which these conclusions were based shows, however, that the few apparent crossovers there observed may really have been caused in some cases by the phenotypic overlapping of the genotypic classes present, and in other cases by non-disjunction.

instead of by crossing over. In the present experiment, where such errors were guarded against, no evidence of any crossing over appeared, either between e_y^D and bent or between e_y^D and M_{IV} , and the conclusion may thus be drawn that crossing over probably does not occur between the tiny fourth chromosomes, and that a valid genetic map of their contained genes cannot be made by the crossover method hitherto employed.⁸

The significance of the findings concerning e_y^D for the present work lies however in a different direction: namely, in the contrast which appears between them and the already published findings of BRIDGES concerning the effect of a total loss of one of the fourth chromosomes. The "haplo-IV" individuals in which, through some mitotic abnormality occurring in a previous generation, one of the fourth chromosomes is completely missing, have been studied by BRIDGES in considerable detail, and their composition has been conclusively demonstrated both cytologically and genetically. Phaenotypically, such individuals show various abnormalities, as would be expected owing to the abnormal proportion existing in them between the number of genes of each kind in chromosome IV and the number in the other chromosomes. The most conspicuous abnormality is the reduction of size of the bristles; less striking are slight changes in wing shape and body build. But no reduction whatever in size of the eyes, or unevenness in their contour, is discernible, nor are the sex combs enlarged. The known loss of all the genes in one of the two fourth chromosomes, including the loss of the normal gene at the locus of e_y^D , thus produces no such effect as is found when the latter normal gene in one of these fourth chromosomes "mutates" to e_y^D (the other fourth chromosome in each case remaining normal). Does not this indicate that the mutation of the normal allelomorph to e_y^D is not a loss?⁹

There is only one possible objection to the conclusion above suggested, and though it does not seem a very plausible one it cannot be definitely

⁸ Since the above was written, we have noted a reference to a somewhat similar test made by BRIDGES with M_{IV} , with similar results (see MORGAN, STURTEVANT and BRIDGES 1926).

⁹ By a curious coincidence, the recessive eyeless gene has sometimes been especially pointed out as a good example of a mutation resembling in its effects, and possibly consisting of, a loss. The conclusion was based on the fact that certain haplo-IV flies, carrying eyeless (ey) in their only fourth chromosome, were found, which seemed of a more extreme eyeless type, it being reasoned that since the loss of one fourth chromosome seemed to increase the "eyelessness," the gene for eyeless itself might be of the nature of a loss. This explanation disregarded the fact that the haplo-IV condition, even in the presence of all normal genes, produces various phaenotypic abnormalities, and so it would be not surprising if it affected the variable eye size of "eyeless" flies in one direction or the other. The evidence of the above text, indicating that not even the more extreme allelomorph, ey^D , is probably a loss, now makes it likely at the same time that ey is not a loss either, but rather the product of another kind of change in the inner composition of the gene.

laid aside. That is, finding the effect of a loss of the whole of the fourth chromosome is not a perfect test of what effect the loss of a single gene in the chromosome might have, for the loss of the other genes at the same time might somehow exactly compensate for the effect of the loss of the particular one in question. That would be equivalent to saying that the effect (the so-called "eyeless" condition) was really due to the disproportion ("unbalance") of gene-quantities arising between the normal gene (E_v) at the locus of e_v^D and the other genes in the fourth chromosomes, when one E_v gene was lost, rather than to the genic disproportion then arising between the number of E_v genes and of genes in the other autosomes: that is, the intra-fourth-chromosomal genic disproportion in this case would have to be much more important than the inter-chromosomal genic disproportion. Since, however, the other autosomes probably contain at least a hundred times as many genes as the fourth chromosomes, the chance of the intra- rather than the inter-chromosomal disproportion being the source of the eyeless condition would, other things being equal, probably be less than one in a hundred.

It is true that, in the case of the X-chromosome, we have found the effect of inter-chromosomal disproportion to be relatively small, as compared with that of intra-chromosomal disproportion, but the reason for this is evident in the history of the X-chromosome, since the rest of the genetic complex has had to become adjusted to the presence of the X in either one "dose" or two without giving rise to abnormal phaenotypic manifestations. There could not very well have been such a process at work in the case of the fourth chromosome (unless it be supposed that part of the fourth chromosome has been derived from the X by a translocation). Hence it seems on the face of it far-fetched to ascribe the observed phaenotypic effect of " e_v^D " to a disproportion involving the relatively few genes in the fourth chromosome itself, rather than to that involving the hundred or so times as many genes in the other autosomes.

If, however, the genes directly adjacent to a given locus commonly exert an important effect on the expression of the gene at that locus, through some reaction dependent on their contiguity (as suggested by STURTEVANT's work on Bar eye, 1925) then this would be a factor tending to make the effect of intra- as compared with inter-chromosomal disproportion much greater than it otherwise would be. On such a hypothesis the "eyeless" character could be formally explained, as the result of a loss at the " E_v " locus, with a consequent effect on the mode of manifestation of the immediately neighboring genes, and the loss of the whole chromosome, by removing these latter genes also, would then fail to pro-

duce such an effect. All this, of course, is a very "special" hypothesis, having little known factual background, but since there is at present no way of disproving its possibility, or even of readily evaluating its plausibility, it must be admitted that the case of dominant eyeless does not, in itself, furnish final proof of an induced "point mutation" not involving the loss of a gene. It should also be pointed out that since this mutation has been observed only once (though a number of similar looking cases were not tested out), the evidence for its having been induced by the treatment remains incomplete.

VI. FIRST ATTEMPTS TO INDUCE MUTATIONS IN BOTH OF TWO OPPOSITE DIRECTIONS

In addition to the above lines of rather indirect evidence converging from various angles, it was felt that it would be desirable to have some really clear-cut experimental evidence which would be capable of proving more definitely and directly, and with as little theorizing as possible, our contention that the induced mutations did not all consist of mere breakdowns, losses, or displacements of the genes. The difficulty usually encountered in arriving at any such evidence lies in the fact that the outward character can in itself give no clue concerning the nature of the gene which is responsible for the effect seen. The absence of a somatic structure does not imply the absence of a gene, nor does recessiveness necessarily imply absence, and dominance, presence, as was once claimed. Fortunately, however, there is a rather simple short-cut attack possible on our present problem, not necessitating the acquisition of any further knowledge concerning the complicated chemical processes whereby a gene attains its phaenotypic expression. The method in question consists in determining whether or not mutations can be induced in both of two opposite directions. The bearing of the occurrence of reversible mutations on the general problem of mutation by loss was discussed by MORGAN in 1913 and again by MULLER in 1921 (MULLER 1923). SAFIR (1920), MORGAN, BRIDGES and STURTEVANT (1925), and especially TIMOFEEFF-RESSOVSKY (1925, 1928) have since given us additional examples of its application, in the case of "spontaneous" mutations. It is only necessary for us here to apply this same idea to our present more specific problem of mutations induced by irradiation.

For the purposes of this method it may be granted in advance that in any given instance we can never determine whether or not the induced mutation under consideration consists of a loss, partial or complete, of a gene. Even if it be admitted to be a loss, if we can then take the resultant

mutant, and, by treating it, cause it to mutate back again to the original form, we have, in effecting the latter step, caused a change opposite to the loss, which must therefore have been a gain of some kind. Contrariwise, for all we know, it may have been the second step, the reverse mutation, which was the loss, but in that case, by the same reasoning, the original mutation must have been a gain. It is also possible, and, in the authors' opinion, more likely that neither of the two opposite reactions were losses, but that both involved substitutions or rearrangements of parts, that were reversible in character, after the manner of many chemical alterations. Certainly it is difficult to conceive of either of the mutations as a complete loss, since if it were it would scarcely be expected that at another time the same gene as that which previously was present at that locus would somehow become suddenly recreated. But, no matter which of these interpretations were really correct, it would none-the-less be clear that *both* of the opposite mutations could not be losses, and so the demonstration that both were really induced by the irradiation would settle the major question at issue, namely, that mutations which were not of the nature of losses could be induced. While a positive result would thus lead to a positive conclusion it must be borne in mind that a negative result (lack of success in being able to induce both opposite mutations) would not prove the negative conclusion, that the mutations which occurred were necessarily losses.

In a first attack on this question, it was thought desirable to be able to study the possibility of mutation in respect to a number of genes in each individual examined, so that the chance of finding some gene or genes that would respond in both directions might be increased. The so-called "IIIpl" stock was used for this purpose. This contains the following genes, all located in the third chromosome, in the order given: *r_w* (roughoid eye), *h* (hairy), *s₁* (scarlet eye), *p* (pink eye), *s₂* (spineless), *c* (ebony). These six genes are all sufficiently independent of one another in their expression that a change from any one of them to its normal allelomorph would be readily noticeable in a culture of IIIpl flies, and a change from the normal to any of these mutant characters would be readily noticeable in a culture of normals. In order to be able to detect the changes in both directions, it was necessary to treat both opposite types of flies—the non-IIIpl's¹⁰ and the IIIpl's. Treated adult males were used, as these can be given a stronger dose than the females or immature individuals without becoming sterilized; they were given the heavy t4 (old machine) dose on October 26, 1927. The treated IIIpl males were immediately

¹⁰ The males used here which contained the normal allelomorphs of IIIpl contained the dominant Curly wing in one of their second chromosomes.

crossed to untreated IIIpl females, and the progeny were examined carefully for flies showing any of the normal allelomorphs of the characters concerned. Any of these normal allelomorphs would manifest themselves if they were present, since they are all dominant to the recessive allelomorphs that would be received from the untreated parent. The treated "non-IIIpl" males were likewise crossed to (untreated) IIIpl females, because the mutations of the normal genes from these males would be expected to produce recessives that would be able to manifest themselves only if recessive mutant allelomorphs were received from the female parent as well.

In all, there were 2,318 offspring from the cross of IIIpl by IIIpl. Only two of these aroused any suspicion that they might contain a mutation of one of the genes in question to or towards its normal allelomorph. One was a female that looked as if it might possibly contain a non-pink gene, but which proved, on testing, to be germinally a pure IIIpl, and the other was a male whose eyes appeared somewhat non-roughoid, but which proved to be sterile. There were various other mutations, most notable among which was a Notch-wing female which proved to carry the "mottled-1" eversporting allelomorph of white, combined with a translocation, that has been described elsewhere (MULLER 1928c, 1930).

The cross of treated non-IIIpl Curly males by IIIpl females yielded 2,170 offspring. Among these there was one Curly scarlet female which, when crossed again to IIIpl, transmitted the scarlet character. Later tests proved the new scarlet to be non-lethal but in close proximity to a lethal. Another Curly female showed the spineless character, and transmitted it; the new spineless was allelomorphic to the old, and, like it, to aristopedia; but it was a lethal. A spineless-appearing male failed to transmit his variation, and another male, part of whose body was similar to spineless, failed to leave offspring. There were five flies having eyes of a somewhat rough appearance; of these, four were fertile and none of them proved to contain in their treated chromosome a mutation to roughoid. Three of them contained, instead, genes resembling Star eye, and of the latter, one was tested sufficiently to show that it was really at the locus of Star (in chromosome II) that the mutation had occurred; the fourth, in which only one eye suggested roughoid, the other being normal, transmitted no visible variation but proved to have a translocation involving chromosomes II and III. There were, in addition, other visible variations observed among the offspring of the treated non-IIIpl males, but none involving the characters of IIIpl. Altogether, then, there were two certain mutations (or losses?) involving the loci in question—one scarlet and one spineless.

It might also be mentioned that 376 offspring were examined from a cross of similarly treated Curly but otherwise normal males by females homozygous for a_r , p_x , s_x in chromosome II and t_h , s_r , e_r , r_o , c_a in chromosome III. In this cross, no mutations in the loci in question were observed, except for one possible arc winged (a_r) female that was sterile.

Since no "reverse mutations"—from the mutant to the normal type—had been observed in the above experiments, the point at issue still remained unproved. The work was then temporarily discontinued, to allow of certain other experiments, it being planned to continue such tests later, using other loci. The work had shown that some of the characters of IIIpl had certain disadvantages in detection, hairy and roughoid both requiring considerable care, and roughoid and spineless being easily confused with non-allelomorphic dominant variations that occurred rather frequently. Since then, TIMOFEEFF-RESSOVSKY (1929 c) in independent experiments has used the same method successfully (see p. 568).

In the winter of 1927-1928, Doctor F. B. HANSON examined in our laboratory a considerable number of progeny from irradiated $y\ w\ f\ B\ B_x$ males crossed to untreated females containing attached X's, with a view to the discovery of possible reverse mutations. It may be recalled here that they showed 4 reverse mutations of B (Bar-eye) to non- B , and no reversals in the other loci, in a count of 4,662 male offspring (866 from a t_8 and the rest from a t_4 raying) (HANSON 1928). These results were of considerable interest in connection with STURTEVANT's work on reversals of Bar (STURTEVANT 1925). Unfortunately, from the standpoint of the question at issue in our present paper, the opposite change, non- B to B , has not been observed as yet in any irradiation experiments, so that the question of loss versus other change still remained undecided. It seemed evident that very large counts were needed if positive results were to be obtained.

As a third step in the attack, one of the authors (MULLER), in the spring of 1928, undertook to irradiate the larval stages of white-eyed flies in order to see if any that hatched showed pigmented ommatidia. It had previously been shown (PATTERSON 1928) that when the larvae of red-eyed flies are irradiated, gene-mutations to white occur in some of the cells destined to form ommatidia, so that individual white facets or groups of them are found in the adult eye (the number in the group depending upon the stage of cellular subdivision of the eye anlage at which the treatment occurred). Thus, for the purpose of our present problem, it was only necessary to prove that the change from white to or towards red could be induced likewise. Such proof assumes that in a stock of white the appearance of pigment (which could of course not be subjected to the breeding test)

would necessarily be due to a mutation at the locus of white, but this would be highly probable since of all the numerous eye color mutations known at other loci in *Drosophila*, none have been found to cause any production of pigment when the gene for white was concomitantly present.

If large counts were the desideratum, there was a distinct advantage in using the present method, even though only one locus was under observation. The advantage lay in the fact that each group of facets derived from a single cell present in the eye at the time of treatment would show mutations independently of every other such group of facets. If the treatment was given at such a late stage that nearly every cell then present in the optic rudiment represented a separate ommatidium, and yet early enough for the effect still to be producible in most of the ommatidia, the majority of the facets would be independent in their mutations of the other facets, and would manifest their mutations independently. This optimal stage, as previous work had shown, was when the larva was between 3 and 4 days old (after the egg had been laid and kept at a temperature of 27° C).

Since there are on the average about 850 ommatidia in each eye, the examination of both eyes of one fly should therefore reveal the number of mutations to white occurring in something like 1700 separate elements—a result as significant as if so many separate flies had been tested for germinal mutations. In the previous work on mutations from red to white, it had been found by PATTERSON that there was about one such mutation in 10,000 elements (containing one X chromosome), when an average dose of approximately t4 was applied to somatic cells. This is equivalent to 1 in 3,300 for the t12 dose. Considering the different conditions of the experiment this is not so very different from the figure, 1 mutation to white in 1,000 with the t12 dose which we have seen was found for germinal mutations induced in sperm cells. If now there were a frequency of reverse mutation from white to red in the larval somatic cells similar to that from red to white (1 in 10,000 for t4) then there should be an average of about 1 red ommatidium observed in every 3 male flies examined, when the larvae had been treated with a t8 dose (the one used). The female flies from treated larvae of homozygous white stock should show red facets twice as often as this, since there are two X chromosomes in each cell of the female, the gene for white in either of which could mutate to red and, as a dominant, manifest itself independently of the other. Thus there would be something like two-thirds as many red facets found as flies observed, in the case of females, and in the total population there would be about half as many red facets as flies. (If some of the cells at this stage still represented

groups of facets, or if some were at too late a stage for the production of an observable effect, the number of cases of red facets observable would be correspondingly lowered, but probably to not less than half the above number, in view of existing data concerning the stage in question.)

The stock of white used first was one designated as *wvf*, in which the white had itself been produced by X-rays, being the mutation shown in in line 4, table 4. The object of using such a white was to test out whether the white of X-ray origin was itself reversible, for it was conceivable that this particular kind of white might be a loss and really different from whites of spontaneous origin. Vermilion (*v*) which was in this stock could not interfere seriously with the detection of a mutation in the white locus, while forked (*f*) was useful as a check against contamination.

A large number of flies of this stock were allowed to lay their eggs, during 24 hours, on a flat circular slab of banana-karo-yeast-agar of the same diameter as the field under the X-ray machine. Ninety-six hours after the parents had been placed on this food (and 72 after they had been removed) the slab was taken from the incubator at 27° C where it had been kept, and subjected to the t8 dose.¹¹ When the progeny had hatched both eyes of every fly were carefully examined under the high power of the binocular in the search for red or reddish ommatidia. Four hundred fifty-one were studied in this way, consisting of 232 females and 219 males. Multiplying the females by 2 in order to allow for their two X's and then multiplying the figures for both females and males by 1700 (the approximate number of facets per fly) we see that a total of about 1,160,000 X-chromosomes (or at least half that number, in view of the qualifications in the last paragraph but one) were here studied for the mutation of white toward red. Among them a few cases were found of isolated discolored (grayish or reddish) facets which, when studied under the high power of the compound microscope, proved to be due, not to red or yellow pigmentation of the cells of the ommatidium which normally are colored, but to an opacity of the lens forming the surface of the facet; these then were not representative of the phenomenon which was being sought for. There was in addition one male that showed a group of four discolored facets which appeared to be of just the same type as that which has just been described, but which was lost before the examination under the high power was completed. There were no other cases that even suggested a reverse mutation.

If reverse mutation had been as frequent as the red-to-white mutation

¹¹ In another publication (PATTERSON 1929b) the dosage in this experiment was reported as "D5" instead of t8 and the age as 64-72 instead of 72-96 hours.

has been found to be, from one to two hundred colored ommatidia would have been found. Thus, in spite of the comparatively small total number of individual flies observed, the multiplicity of ommatidia allowed us to be certain that the frequency of this reverse mutation, if it could occur at all, must be far lower—something like a hundredth as great, at most—than that of the mutation in the direction red towards white. This was somewhat surprising in view of the fact that there is a spontaneous mutation of the kind in question already on record—namely, a mutation of white (itself of spontaneous origin) to eosin, found by MORGAN (MORGAN, STURTEVANT, MULLER and BRIDGES 1915, 1923). Cases were also on record of individual red facets arising spontaneously in stocks of white eyed flies (SPENCER 1926). SAFIR in 1920 reported red arising from eosin. It seemed however, that for our present purposes still larger numbers or a still different technique would be desirable.

Subsequent to the above experiment, considerable numbers of flies carrying white or some mutant allelomorph of white—tinged, eosin, apricot—have been treated by PATTERSON at one or another pre-pupal stage, and examined after emergence. In all, among 1040 white, 245 tinged, 2424 eosin, and 501 apricot flies from experiments suitable for the present purpose, treated with doses of D4 to D10 at various stages of their larval life, there was just one case in which a darker color appeared on a lighter background. This was in an apricot male and will be referred to later. The darker color in question was not as dark as the normal red. It will be recalled that apricot or an allelomorph very similar to it has itself arisen from red in an irradiation experiment reported in an earlier section.

As will be seen on page 568, TIMOFEEF-RESSOVSKY, independently using the method of treating white and eosin flies in larval stages, has obtained a case of red facets in an otherwise white eye, another case of reddish (but not red) facets in a white eye, and one case of red facets in an eosin eye.

VII. REVERSE MUTATIONS AT THE LOCUS OF SCUTE.

In the section on multiple allelomorphs, evidence has been presented to show that the normal gene at the locus of scute can be caused to mutate to scute by means of X-rays. Before this evidence was obtained, results had been secured in an experiment having a different primary object, which indicated strongly that the opposite change also could be induced, namely, from scute back to the normal, non-scute (MULLER 1928 a, c).

The object of the experiment had been to ascertain whether induced mutations occurred in only one or in both members of a pair of allelo-

morphs present in a treated cell. In order to discover this, it was necessary that both members should be transmitted together to the next generation, and then tested; in other words, they had to undergo non-disjunction. A stock was therefore used which was known to have a strong tendency towards non-disjunction. The females of this stock contained in one of their X chromosomes the combination $s_c C_l B s_m v t$ (the order of the genes beyond scute is normally the reverse of this) and in the other X chromosome the genes $s_c v f b_b$. C_l indicates a lethal inversion; hence the abnormal gene arrangement in the chromosome containing it. Since the presence of this must prevent a complete point for point apposition between the X chromosome containing it and the other X, having the normal gene arrangement, there is a tendency to imperfect pairing and to resultant non-disjunction. It was therefore not difficult, in outcrosses of such females, to find some cases of primary non-disjunction in which both X's had been received from the mother and a Y from the father. These F_1 non-disjunctive females would then, on account of the presence of the Y, exhibit secondary non-disjunction; the latter would be particularly high in frequency because of the non-matching of the X's and the presence of b_b heterozygously.

A single Y-containing female of the composition just described was used to start the experiment proper. It was treated with the t2 (old machine) dose on October 26, 1927, and immediately crossed to $y^2 b_b$ males, being transferred through two cultures. From the count of progeny, given in table 5, line 1, it can be calculated that the two X's and the Y segregated at random from each other, with no preference to any particular kind of pairing and disjunction. It is also evident from the results that there was no lethal in the $s_c v f b_b$ chromosome of the mother, since the males bearing this chromosome are viable.

Forty F_1 non-disjunctive females from the above cultures, which necessarily had received both X's from their mother, were then tested for sex-linked lethal and other mutations, those which were certainly virgins being mated by $y^2 b_b$ males (the parental cross thus being repeated) and those of doubtful virginity by S/C_v males, whose dominant genes would make their progeny recognizable. In all 40 cases "regular" (not non-disjunctive) sons appeared, bearing the $s_c v f b_b$ chromosome; hence no new lethal had arisen in this chromosome. Nor did any newly arisen visible mutations make their appearance. The work was therefore carried further. Six of the above 40 F_1 females had themselves been irradiated with a t2 treatment from the new machine. Their progeny would therefore serve as well for testing as would that of the original female used

TABLE 5

Progeny of non-disjunctional females having (except for newly arisen mutations) the composition $\frac{s_c C l B s_m n}{s_c s f b b}$ crossed by $\frac{y^b b}{Y}$ or $\frac{S}{CY}$ males.

COUNT NO.	MOTHER OF FEMALE	DESIGNATION OF FEMALE	PECULIARITY OF FEMALE	COMPOSITION OF MALE USED	NUMBERS OF OFFSPRING					
					FEMALE			MALE		
					Regular		Non-disjunctional	Regular	Non-disjunctional	
					+	B	(<i>s_c</i>) ⁺ <i>s</i> B	triple-X	(<i>s_c</i>) ⁺ <i>y</i>	<i>y</i> ^b (or +)
					and <i>b_b</i>					
1.	Primary non-disjunctional	Original P ₁ ♀	Irradiated	<i>y</i> ^b <i>b_b</i>	41	56	45	0	41	53
2.	Original P ₁ ♀	F ₁ ♀ <i>a</i>	"	"	31	42 (1 <i>b_b</i>)	21	0	25	26
3.	"	" <i>b</i>	"	"	34 (1 <i>A</i>)	25	19† (1 <i>A</i>)	1	22	29 (1 <i>A</i>)
4.	"	" <i>c</i>	"	"	10	5 (1 <i>A</i>)	2	0	9	5
5.	"	" <i>d</i>	"	"	44	30	15	0	38	25
6.	"	" <i>e</i>	"	"	56	65 (1 <i>M</i>)	48	3	41 (1 <i>A</i>)	52
7.	"	" <i>f</i>	"	"	27	17 (1 <i>A</i>)	15	0	20	15 (2 <i>A</i>)

TABLE 5 (continued)

8.	F ₁ ♀ a (br.2)	F ₁ ♀ a	s _c -reversal (somatically nearly non-s _c)	S — C _y	16 (1M)	19	19 (all non-s _c)	0	20 (all non-s _c)	29
9.	F ₁ ♀ b (br.1)	" b	s _c -reversal 2 (somatically s _c)	y ² b ₆	4	1	6 (one non-s _c)	0	5	7
10.	F ₁ ♀ c (br.2)	" c	lethal 1	S — C _y	16	24	35	0	0	38
11.	F ₁ ♀ d (br.2)	" d	lethal 2	"	4	4	8	0	0	4
12.	F ₂ ♀ d	F ₂ ♀ a, b, & c	lethal 2		21	26	30	0	0	30

* Scute except where non-scute is indicated.

† Numbers in parenthesis refer to aberrant forms included in count above parenthesis; A, phenotypically abnormal in some way; M, minute bristles.

† Many offspring lost from this culture after examination but before counting.

(except for the unintentionally smaller dosage of irradiation used). To show that they themselves carried no new mutant gene, the counts from each of the six (which had been crossed to y^2b_h) are given in table 4, lines 2 to 7. (As in all F_1 from irradiated parents, various isolated abnormalities appeared; most of them are simply marked "A" in this table.) It will be seen that the results are similar to those from the first female. Sixty-four of the non-disjunctional females ("F₂") from these latter cultures were then again tested as before (virgins crossed to y^2b_h ; others to S/Cy). It was among these cultures that the mutations of interest were found.

TABLE 6

Tests of lethals recorded in table 5. Cross: $\frac{s_c v f b_h}{Y} \text{ } \varnothing \times + \text{ } \sigma^7$ (l_1 between v and f ; l_2 between f and b_h).

COUNT NO.	DESIGNATION OF MUTATION	NUMBER OF OFFSPRING								(n-d) +
		Females (all)	Males							
			<i>s_cv_f</i>	<i>s_c</i>	<i>v</i>	<i>s_cv</i>	<i>f</i>	<i>s_cf</i>	<i>v</i>	
1	lethal 1	51	0	11	2	4	0	0	0	8
2	"	40	1	2	0	2	0	0	2	8
3	"	49	0	6	2	4	0	0	0	14
4	"	52	0	10	2	4	0	0	0	12
Sum	"	192	1	29	6	14	0	0	2	42
5	lethal 2	44	0	13	0	1	0	2	1	7
6	"	86	0	8	0	1	3	0	1	15
Sum	"	130	0	21	0	2	3	2	2	22

There were, among these cultures, two cases of lethals in the $s_c v f b_h$ chromosomes. These are shown in lines 10 to 12, table 5. They must have been newly arisen, since in the mothers, counts from which are given in lines 4 and 5, respectively, they were not yet present. The tests given in table 6 show that they were different from one another, the first found being located about 6 units to the right of forked, and the second about half way between vermilion and forked. These lethals could not have originated simultaneously in the $C_1 B$ chromosome also, for in that case the non-disjunctional females of lines 10 and 11, table 4 would have been homozygous for their respective lethals, and would not have lived to maturity. There was thus no escape from the conclusion that these mutations had occurred in only one of the two homologous chromosomes present in the cell at the time of treatment, and the original objective of

the experiment was attained. In addition, however, to this result, which is aside from the theme of the present section, there was another finding, or pair of findings, quite unexpected and surprising at the time, which is of importance to us here.

The counts of the two cultures in which these findings were made are shown in lines 8 and 9, table 5. In both cases a reverse mutation from scute to non-scute had occurred. It is quite evident, moreover, that the two cases represent independent mutations, since in the first one (line 8) the non-scute is inherited in the non-Bar chromosome, and thus appears in the males, whereas in the second, the results shown in line 9, when coupled with later tests (table 7, cross III), show that the non-scute is in the C_1B chromosome. (Later tests on the C_1B chromosome of the first case proved that scute was still contained in it.)

The original (F_2) mutant female of the first case was still present with the F_3 in the culture of line 8, and was examined to see why she had not been recorded as a non-scute. It was found that she was in reality non-scute, phaenotypically, except in that she lacked one bristle on the scutellum and might hence have passed as a "minus" (towards normal) variant of scute. The mother of the culture of line 9 was also found, and she was seen to be a typical scute; this agreed with the fact that most of her Bar offspring were likewise scute. The latter case thus provided an illustration of the fractional effect (the first found from treated female cells): the C_1B chromosome in the treated cell was evidently split already at the time when the treatment was applied, so that part of the body, including much of the epidermis and germinal tissue, came to have an unmutated C_1B chromosome, and the rest received the newly mutated C_1B chromosome. This result, by showing how late in the germ cell history the mutation had occurred, also gave further proof that the two reverse mutations must have been independent of one another.

Although we have in the above account referred to these mutations as "reverse mutations from scute to non-scute," the data so far given do not preclude the possibility that one or both of them had occurred in some other locus than that of the familiar gene for scute. The mutant genes might, in other words, have been dominant "suppressors" of scute, non-allelomorphic to the latter, instead of simply dominant normal allelomorphs of scute. Their method of inheritance showed them to be sex-linked, but the data did not yet show in what locus they lay. If they were "suppressors," the chromosomes containing them still contained the original gene for scute at its usual locus, but contained, in addition, the suppressor (an abnormal gene not present in wild type flies) at some other locus. To

decide between these two possibilities, it was necessary to study the linkage relations of the new non-scute.

In order to test these linkage relations, a cross was made between stock of the first non-scute ($S_e^{#1}$) and flies from a stock of yellow scute (ys_e). The heterozygous females, containing in one X the combination of "non-scute" and ηfb , and in the other X, ys_e , were back crossed to ys_e males. The count of 357 flies is given in table 7, "cross I." If the non-scute chromosome had really contained s_e and a suppressor, then the females tested here would have been homozygous for s_e but heterozygous for the suppressor, and the count of scutes versus non-scutes would have reflected entirely the distribution of this suppressor; its linkage relations (locus) would therefore be disclosed directly by the crossover ratios. The table shows that scute here is linked completely (so far as these numbers can show) with the locus of yellow, just as scute ordinarily is. The lack of crossing over with yellow is, however, not due to any abnormal reduction of crossing over between the X chromosomes, since y , v , and f cross over in quite normal fashion. It must accordingly be inferred that the mutation, if not in the locus of scute itself, was so close to it that no crossing over between these loci occurred in a count of this size.

A second test of the first scute reversal is recorded in table 7, "cross II." This test was essentially similar to the first, except in that the true normal allelomorph of scute was present in the chromosome with yellow, instead of scute itself. Since the locus of this gene is, in all ordinary crosses, inseparably linked to that of yellow it would not be expected that any of the yellow offspring from this cross could show scute, no matter what sort of a mutant had been present in the non-yellow chromosome. The non-yellows were therefore not counted. The yellows were as useful for our purpose as the flies in "cross I." They would have contained s_e if the suppressor hypothesis had been correct, and those of them would have shown the scute character in which this suppressor had crossed over from s_e , leaving the latter to manifest itself. The count of 251 (yellows) again failed to show any crossovers between the new mutant gene and the locus of s_e , and the hypothesis of non-allelomorphism was thus made exceedingly improbable.

The determination of the locus concerned in the second case of apparent reversal presented the difficulty that the mutated gene was located in the C_1B chromosome, which, owing to the inversion in its right hand region, undergoes very little crossing over with other chromosomes. There is, however, very rarely a single-crossover near the left end of the chromosome and also an occasional double-crossover in regions further to the right.

As the small sample count in table 7, case III, shows, only non-crossovers are ordinarily obtained. In the attempt to obtain a crossover of the desired composition, over a thousand flies from crosses of this type were examined, without exact counts being made. None were found of the composition

TABLE 7
Tests of scute-reversals recorded in table 5.

$$\text{Cross I: } \frac{S_c^{x1}vf b_b}{y s_c} \varphi \times y s_c \sigma^7.$$

FEMALES		MALES								TOTAL
+	y _{sc}	CROSSEVERS IN REGIONS DESIGNATED BY NUMBERS								
		0	0	1	1	2	2	1,2	1,2	
		v _f	y _{sc}	+	y _{sc} v _f	v	y _{sc} f	f	y _{sc} v	
110	99	35	45	19	18	19	7	2	3	357

$$\text{Cross II: } \frac{S_c^{x1}vf b_b}{y^s b_b} \varphi \times y s_c \sigma^7.$$

Numbers of non-yellow offspring (yellows not counted but all observed to be non- s_c .)

FEMALES	MALES				TOTAL
+	CROSSEVERS IN REGIONS DESIGNATED BY NUMBERS				
	0	1	2	1,2	
	<i>vf</i>	+	<i>v</i>	<i>f</i>	
148	47	38	8	10	251

$$\text{CROSS III: } \frac{S_c^{x2} C_1 B s_m vt}{s_c vf b_b} \varphi \times y s_c vf u_b \sigma^7.$$

NUMBERS OF OFFSPRING			
FEMALES		MALES	
$s_c v b_b$	$s_c B$	$s_c vf$	OTHERS
10	36	28	0

especially desired, containing the non-scute (or scute suppressor) without the $C_1 B$, which would have enabled extensive crossover tests of the locus involved to be carried out in later crosses. Nevertheless, two females of the contrary class were found, which contained in the chromosome with $C_1 B$ the gene for scute and no dominant non-scute. Tests showed that $t(\tan)$ and s_m (small wing) were still present in this chromosome, so that

the result had not been produced by double crossing over but rather by single crossing over occurring near the left end of the chromosome. This proved that the dominant non-scute which had passed across must be located in this left hand region, that is, very close in the genetic map to the locus of scute itself. Taken in connection with the more extensive evidence from the other case, it thus became highly probable that a real reverse mutation had occurred here too.

Were these reverse mutations of scute really due to the X-rays? There had been no controls to this particular experiment, but scute has been bred extensively, both in homozygous form and also in crosses in which no crossing over from other markers (non-yellow; sex) can occur; if anything like the rate of mutation indicated in the present experiment were common without treatment, numerous such reversals should have been found in this previous work. For the two cases here discovered to have arisen in a total of only 104 F_1 flies tested was a surprising result for a single gene, even in an X-ray experiment. However, in all the previous experiments not involving X-rays, there have been a few positive cases of reverse mutation of scute observed, and while it seemed highly unlikely that two such spontaneous cases would ever be found in a count as small as a hundred, nevertheless apparent "runs" or "epidemics" of certain spontaneous mutations—maroon and purple (BRIDGES 1918, 1919), yellow (PATTERSON unpublished)—have at times been encountered (see, too, BAUR'S finding of "premutation" in *Antirrhinum*) (BAUR 1926). In view of this source of uncertainty, and of the fact that, when the present reverse mutations were found, no induced mutations in the opposite direction—non-scute to scute—had yet been observed, in spite of a rather considerable body of data, it was decided to continue the search for opposite mutations, using by preference some other character than scute.

VIII. A REVERSE MUTATION AT THE LOCUS OF FORKED

Several months later, in the course of another experiment which was being undertaken for a different purpose, another finding pertinent to our present problem was unexpectedly made. This, in turn, furnished a clue suggesting a profitable direction for further research on the subject, which, when followed up, finally led to the obtaining of really convincing evidence of the type desired.

The experiment which served as the starting point was primarily concerned with the securing of mutations in a special type of X-chromosome known, on account of the number of the culture in which it originated, as the "delta 49" chromosome. This chromosome contains an induced non-

lethal inversion in its middle region, and it was desired to secure mutations in it in order that the sequence of its loci might be subjected to study. To avoid the fractional effect which is produced when spermatozoa are treated, whereby many progeny are found which are somatically mutant

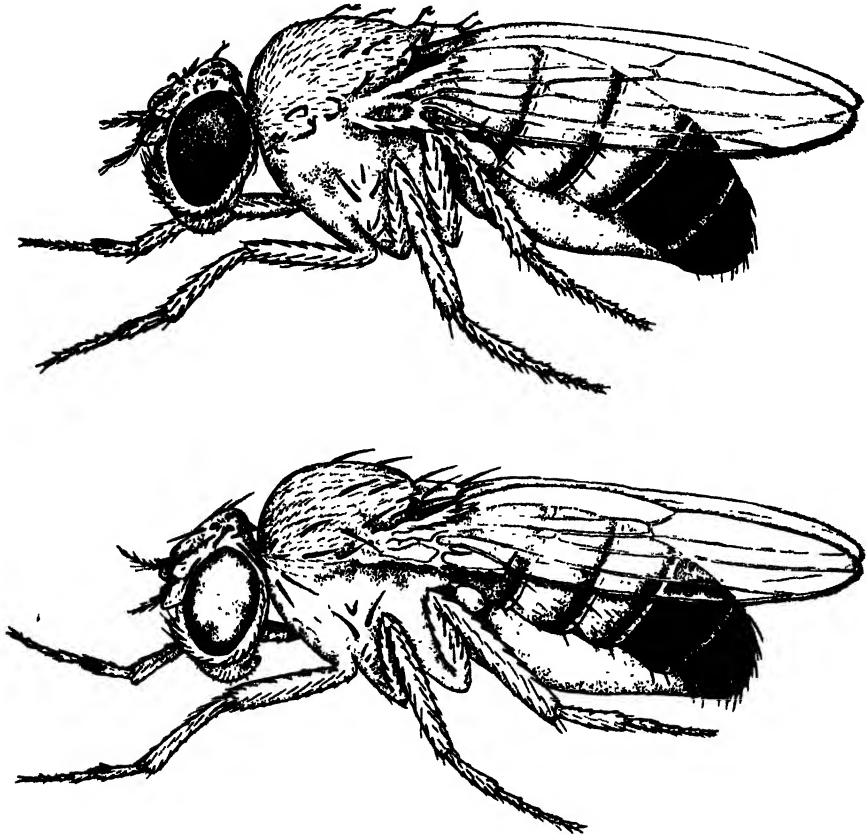


FIGURE 3. Above, forked-bristle fly with normal eye. Below, normal-bristle fly with "spectacled" eye, arising (after irradiation) from a race like that shown above. The normal bristle represents a *reverse* mutation of the mutant gene for forked to non-forked bristle. This is a case of double mutation.

but fail to carry the mutant gene in their germ cells, it was decided to treat larval stages. In flies treated previous to the maturation division, the sorting out of mutant and non-mutant genes into different cells should be completed by the time the stage of the spermatozoon is reached. Accordingly, flies of the $\delta 49f$ stock (containing *f*, forked, to the right of the inverted region) were given the t8 treatment 3 to 4 days after the eggs from which they were derived had been laid (they were kept at 27° C until treated). The male imagos which resulted were then separately crossed in 71

individual bottles to yellow attached-X females. After a week, these parents were transferred to a second brood (culture) and left there for another week.

In this cross, it was to be expected that the sons would show any abnormalities due to mutant genes in the $\delta 49 f$ X-chromosome, since they received this from their father and their Y from their mother. The daughters, not containing the treated X, might be disregarded. From previous crosses of this kind, in which, however, the father had been treated when adult, it was expected that a fairly high frequency of transmissible mutations (one-half to one percent) would be detected in the sons. In all, 2651 male progeny were examined, consisting of 1520 from the first brood and 1131 from the second. Among these there were several dozen with some kind of abnormality, of "nature" or "nurture." Most of these abnormal males were sterile; among the rest, the abnormality was usually slight or traumatic in apparent origin, and proved not to be inherited. In just two male flies was it possible to demonstrate any transmissible variation. One of these variants exhibited a slight disarrangement of the ommatidia, which was transmitted as an autosomal dominant. The other variant, which appeared in a culture different from the first, containing, in addition to it, 32 yellow non-variant females and 31 forked non-variant males, offered a considerable surprise. For it was a variant not in one respect alone, but in two separate and conspicuous respects. For one thing, it had eyes of a peculiar color and morphology, which we have termed "spectacled." Secondly, the same fly, unlike all the other 2650 males, was non-forked. Figure 3 shows above a fly of the forked type used in this experiment, and below, a spectacled non-forked fly of the stock derived by the double mutation from the above forked.

The doubly mutant individual, having lost its "marker," forked, was at once open to the suspicion of having arisen through contamination of the culture. This seemed highly unlikely, since there was no stock in the laboratory having eyes of the peculiar "spectacled" type, but as a double visible mutation in an experiment where so very few mutations at all were being found seemed unlikely also, it was important to reach a sure decision on this question. This was possible because of the inversion which had been present in the " $\delta 49$ " chromosome and which served as an invisible marker. The fly in question proved fertile, fortunately, and was crossed to females containing $s_{c}vf$ in order that a test of the crossover properties of its X-chromosome might be made. Counts of the male progeny of the F_1 females heterozygous for $s_{c}vf$ and for spectacled then showed no crossing over except for a very small amount of single crossing over near the right

end (between *v* and *f*), yielding the expected forked and scute vermilion flies. This result is characteristic of the behavior of the " δ 49" X chromosome when in combination with an X of normal configuration.

To get more specific evidence that the gene-rearrangement in the chromosome containing spectacted was the same as that in the " δ 49" chromosome, spectacted males were then crossed to " δ 49" forked females, and the resulting F_1 females were bred. The F_2 flies in this instance, unlike those in the previous cross, were found to contain a high percent of crossovers between spectacted and forked, of both contrary classes, spectacted forked and wild-type. This showed that the chromosomes of the two parental cultures "matched," that is, had their genes rearranged in an identical fashion. Now, the only δ 49-containing stock in the laboratory, besides the δ 49 forked stock, was one containing bobbed instead of forked. Homozygous females of the spectacted race proved, however, to be non-bobbed, so that the mutant chromosome was not derived from the δ 49 bobbed race by contamination, and the evidence was complete that it had originated from the δ 49 forked race through a double visible mutation.

Crosses with the previously known mutant, "lozenge eye," and with stocks containing other genes allelomorphous to lozenge then showed spectacted to be an allelomorph of lozenge, though, curiously enough, the combination, lozenge-spectacted, proved much more normal looking in our tests than either pure lozenge or pure spectacted was. Homozygous spectacted females were found to be extremely infertile, and yet, unlike most lozenge females, some of them produced a few offspring. In view of its allelomorphism, the symbol for spectacted may be taken as l_1^s .

It was of greater interest for our present purpose to study the genetics of the mutation that had abolished the forked character. Here, as in the case of the scute reversals, there was the possibility that a non-allelomorphic dominant sex-linked suppressor of forked had arisen, rather than a mutation in the locus of forked back to the original normal allelomorph. The test of the question was made by crossing the spectacted δ 49 non-forked flies to flies of the δ 49 bobbed stock. In this cross, F_1 females were produced both of whose X-chromosomes contained the δ 49 rearrangement, and in which, therefore, crossing over between the X's could occur freely. If the bobbed-containing chromosome really carried forked itself, and, in another locus, a mutant dominant suppressor of forked, then by crossing over between these two loci, a chromosome containing forked without the suppressor would be produced. From a backcross of such females, offspring which received such a crossover chromosome from their mother and a Y

chromosome or an X containing forked from their father would thus show the forked character. But if no forked offspring appeared, it would have to be concluded that the "suppressor" could not cross over with the forked locus, that is, that it was none other than the normal allelomorph of forked itself.

The heterozygous F_1 females were therefore backcrossed to forked males. These males were also provided with the gene for tinged eyes (w^t , an allelomorph of white) in order that forked sons produced by non-disjunction might be recognized by this marker and not confused with

TABLE 8

Test of locus of non-forked reverse mutation 1, originating with spectacted in chromosome carrying the 849 inversion.

$$F_1 \text{ Cross: } \frac{\text{"849"} l_a \text{"F"} f?}{\text{"849"} b_b} \text{ } \times w^t f \sigma$$

where "F" = the non-forked mutation. (Problem: is f present along with "F" or has F replaced f , being allelomorph to it? If f is present, crossovers between F and f , showing forked character, will be produced.)

COUNT OF PROGENY (F_1) OF F_1 CROSS					
FEMALES		MALES			
WILD-TYPE	FORKED	REGULAR			NON-DISJUNCTIONAL TINGED FORKED
		WILD-TYPE	SPECTACLED	FORKED	
528	0	273	199	0	4

forked due to crossing over. The counts were continued until 1000 of the F_2 , exclusive of the non-disjunctional exceptions, had been counted. These counts are shown in table 8. It will be seen from inspection of this table that among these thousand "regular" offspring not a single fly showed the forked character. There could be no doubt, then, that a true reversal from forked to non-forked had occurred.

Granted, now, that the mutation had occurred at this particular locus, it still remained to be proved that it had been caused by the irradiation, that is, that its appearance in this radiation experiment was not a mere coincidence. It was difficult to believe that it was a coincidence in view of the fact that no such cases had been encountered by us previously in work with non-radiated forked. The further fact that it had arisen in conjunction with spectacted was suggestive of a microcataclysm, such as an electron passage, but the nearly complete absence of mutations in the other flies of the experiment seemed almost to "prove too much,"¹² and to suggest

¹² The relative scarcity of mutations was evidently connected with the fact that larval stages had been treated instead of the mature spermatozoa, an inference since substantiated by other

that possibly the X-rays had for some reason been ineffective this time. In view of that complication, and of the uniqueness of the case and of the lack of really critical determinations of the spontaneous reversal frequency of forked, it was evidently not at all a foregone conclusion that the radiation had really been the causative agent. What was needed, before the evidence could be regarded as unassailable, was large enough numbers to be sure of the effect, checked by equally abundant and critical controls in which the results were found to form a decisive contrast to those of the treated series. The above experiment did not meet these requirements, as it had been planned with a different object in view and the result in question had been only incidental to it.

A special series of experiments was therefore finally carried out in the months of January to June, 1929, on a scale of sufficient magnitude to allow the determination of the frequency of induced mutations from the mutant to the normal form. In the planning of this work the suggestions afforded by the preceding results were utilized.

IX. PROOF OF THE INDUCTION OF REVERSE MUTATIONS OF FORKED BY X-RAYS

a. Plan of the work.

It was decided that in these new experiments the forked character should be one of those used, since the above work had given us reason to believe that it might be caused to mutate back to non-forked, and since there was even better evidence that the opposite change could be induced (from non-forked to forked). Besides forked, the presence of at least one other mutant character was desirable, so that the latter could serve as a "marker" for the forked, and *vice versa*, in guarding against contamination. Moreover, such a plan would give opportunity to compare the mutation rates at two different loci. Tinged eye, a very light colored allélomorph of white, recessive to the normal red, was chosen for this purpose. It was already known that mutations from red to or towards white could be produced, and that mutations from white to or towards red

tests, although the larval stages are by no means completely insensitive to the irradiation effect. It is still a question whether the scarcity of mutations from germ cells treated when immature is really due to their insensitivity to the X-ray effect or to the non-mutated immature germ cells multiplying at a higher rate than the mutated ones (HARRIS 1929a).

It may also be noted in passing that the coincidence of two of the three heritable mutations having occurred in one individual, out of the 2561, is so great as to make it likely that mutations tend to appear in groups rather than independently of one another. Other cases of induced double mutations also have been found, apparently with greater frequency than would be expected on the basis of a random distribution of induced mutations.

were much less frequent, at best. Possibly, then, this locus would give us different results from the locus of forked. The presence of a certain amount of color in the case of the tinged allelomorph might, however, conceivably provide a somewhat better basis for mutations towards red than existed in the quite colorless white that had previously been tried. In order that the tinged might be used with the forked, a combination stock, tinged forked, had to be made up. We are indebted to Mr. C. P. OLIVER for making up this combination for us.

After it had been determined that reverse mutations could be produced, it was then decided to try to induce mutations back again to the previous condition.

During the course of this series of experiments on mutation in opposite directions 159,070 flies were reared and examined. The experimental lots included 91,405 individuals, derived from 3977 tested flies treated at larval or adult stages. The control lots included 67,665 individuals, derived from 2330 tested, untreated flies.

b. Reverse mutations from treated larval stages

In the first experiments of this series the larval stages were chosen for treatment. This was done because, in this way, the "fractional effect" which follows irradiation of the adult and prevents detection of some germinal mutations could be avoided, and also because the previous reverse mutation of forked had been found in progeny of treated larvae.

The method employed for handling larval stages was the same as that used in other work of the authors, and as it has already been described in detail (PATTERSON 1929b), a brief account will suffice here. About ten pairs of tinged forked flies were placed in one by four inch shell vials containing food on a cardboard spoon. The females readily deposited their eggs on the surface of the food. The spoon was exchanged for a fresh one twice a day, or every twelve hours. The bit of food containing the eggs was removed from the spoon and transferred to a small stender dish, also containing food, and fitted with a gauze cover. The stender dishes were placed in an insect box with tight fitting lid and kept in an incubator run at 27° C until such time as it was desired to give the X-ray treatment. After the culture was irradiated, the food containing the treated larvae was transferred to the half-pint culture bottle and the flies allowed to complete their development at room temperature. The controls for all such experiments were handled in exactly the same manner, except that the X-ray treatments were omitted.

The only exception to this method is the case of about two hundred

culture bottles handled in the month of May. While the work with these cultures was in progress, the outside temperature reached a point slightly above that at which the incubator was operated. The cultures were then removed and allowed to develop at room temperature. It may be added that all cultures from treated adult flies and their controls were run at room temperature.

Practically all of the cultures were given the D5 (= t4.6) treatment. This was applied by exposing the culture to X-rays for twelve and one-half minutes while the machine was operated at 50 K. V. peak, and 10 M. A., at 12 cm distance from the target, with a 1mm aluminum filter interposed. The only exceptions to this are two groups of larvae rayed with D8 dosage (twenty minutes exposure); (table 9), and one group of adult males treated with D10 dosage (twenty-five minutes exposure; table 10). All of the larval stages irradiated were at 72-84 hours of age except one group (table 9, rows four and five) treated at 48-60 hours.

Shortly after the flies began emerging in the culture bottles, they were removed and the males and females placed in separate vials. This was done from time to time until enough individuals were accumulated to make the breeding tests for germinal mutations. The treated flies were then mated, in all cases except one to be noted, to untreated individuals in various combinations. In the first set of these experiments (table 9), the interval between collections was sometimes so long that many of the treated females had already been fertilized by their treated brothers. In all subsequent experiments, efforts were made to secure virgin females, by collecting the flies every eight hours.

In examining the progeny of the treated and control flies for mutations, the following method was used. The etherized flies were carefully examined under the binocular microscope for mutations of the two sex-linked characters involved, and at the same time the males and females were separated into two groups. The flies were again examined when counts of the number of flies in each group were made. This procedure reduces to the minimum the chances of overlooking a mutant fly; for in work of this character it is highly important to detect every case, otherwise it would not be possible to determine with accuracy the frequency of mutation.

The results obtained from the counts of progeny of the tinged forked flies treated in the larval condition are recorded in table 9. The first column states the larval age (in hours subsequent to egg deposition) at which the treatment was given to the parent flies, the second column, the dose employed, the third, the number of cultures (bottles) in which the

treated flies were used as parents, the fourth, the nature of the cross used in the tests of the treated flies, the fifth and sixth, the numbers counted of female and male progeny, respectively, of the treated flies, and the seventh the number of progeny showing reverse mutations of forked to non-forked. In the fourth column, under "nature of tests of treated flies," the character of the treated fly (or flies) is given first, on the upper

TABLE 9

Reverse mutations from forked to non-forked, from flies derived from treated larvae.

AGE OF TREATED LARVAE	DOSE	NUMBER OF BOTTLES OF TESTS	NATURE OF TESTS OF TREATED FLIES	FEMALE OFFSPRING	MALE OFFSPRING	REVERSE MUTATIONS
72-84 hours	D5	39	1 tinged forked female \times 1 tinged forked male	1,454	1,568	2 females; Nos. 2, 3
"	"	44	2 tinged forked females \times 2 tinged forked males	791	943	1 female; No. 4
"	"	58	2 tinged forked males \times 1 X-X female	..	910	0
48-60	"	37	1 tinged forked female \times 1 tinged forked male	1,289	1,414	1 male; No. 7
"	"	14	5 tinged forked males \times 1 X-X female	..	457	0
72-84	"	43	1 tinged forked female \times 2 tinged forked males	2,246	2,632	2 females; Nos. 5, 6
"	"	26	2 tinged forked males \times 2 X-X females	..	785	0
"	"	48	3 tinged forked females \times 3 tinged forked males, all treated	1,090	1,130	0
"	D8	46	1 tinged forked female \times 1 tinged forked male	1,993	2,324	0
"	"	11	5 tinged forked males \times 1 X-X female	..	264	0
Totals		366	834 tested flies	8,863	12,427	6 (5 females, 1 male)

X-X=attached X-chromosomes (each containing the gene for yellow body).

line of each horizontal row, while the character of the untreated fly (or flies) to which the treated fly was crossed is given second, on the lower line. In just one set of cultures, however, shown in the third to the last row, treated flies were mated with each other (three females by three males in each culture) instead of to untreated flies.

With the exception of the set of cultures just referred to, in which treated tinged forked males were crossed to treated tinged forked females, the treated tinged forked males were always crossed to yellow

females having attached X-chromosomes. The male progeny of such a cross carry their father's X chromosome and serve as an index of visible mutations in the latter, while the female progeny, carrying their mother's attached X's and their father Y's, are of no use for this purpose, and hence were not counted. (This is the main reason for the large excess of male over female progeny in the total count.) The treated tinged forked females, on the other hand, were crossed to untreated tinged forked males, and here male and female progeny were examined and counted, as both alike served to reveal any dominant genes that had arisen by mutation in the mother's X.

As the table shows, there were, in all, 834 flies bred which were derived from treated tinged forked larvae. They gave rise to 8,858 tinged forked females, 12,426 tinged forked males, and five females and one male which were *tinged* but *non-forked* and which therefore represented reverse mutations (or else mutant "suppressors" of forked).

No effort was made in this and the later experiments to search the material for other kinds of visible mutations because it was desired to focus the attention on mutations involving changes in eye color and in bristle structure. Nevertheless, many visible changes of other sorts were observed in the large number of flies examined. Some of the conspicuous mutations were recorded, and a few of these were isolated and developed into stocks. The records show that the following familiar variants were observed: apparently white eyes (five times; but these may have included cases of other eye-color mutants, like vermilion and garnet, which, with tinged, would produce a practically white effect), singed, scute (twice), dichaete-like, rudimentary-like wings (eight times), and miniature-like wings. The two mutations to scute have already been discussed.

The fact that five of the reverse mutations were found in the females and only one in the males has little statistical significance owing to the smallness of these numbers. It was to be expected that more cases of reverse mutation were to be found in the female offspring than in the males because the female offspring in many cases had received two treated X's (one from each parent) and therefore had two chances of mutation, whereas the male offspring had but one X in every case. Two treated X's were present not only in all the female offspring recorded in the third to the last row and known to have had both parents treated, but also in a large proportion of the female offspring in the other parts of the experiment. This resulted from the fact, previously stated, that the treated mothers were in many instances not virgin, that is, they had been allowed to mate with their treated brothers before they were placed with the untreated males for the

breeding tests. In consequence, the exact number of treated chromosomes which were present and subjected to the reverse mutation test, in the counts of daughters of treated females, must have been much greater than the total number of these daughters, but less than twice this number, and in any event, the number of these tested chromosomes in the daughters was probably considerably more than the number in the sons. This would, of course, tend to cause the discovery of a greater number of mutations in the female offspring than in the males.

There is also another fact which has some bearing on this point. The two reverse mutations numbered 2 and 3 came from the same bottle, that is, these two females were sisters. Since mutations of any visible kind are rare, the appearance of two identical mutants in the same culture, originating from a single pair of flies, suggests at once that they arose from a common germ cell which divided one or more times after the mutation had been induced. It is not uncommon to find two, or even more, identical mutants arising from a single fly treated during the larval stage (PATTERSON 1928). If this interpretation is correct, the figure 5, representing the mutations found in females, is subject to a greater probable error than that calculated by the ordinary rules of simple independent samples.

The question as to how the frequency of mutations in the treated female larvae compared with that in the treated male larvae is a different one from that of the frequency with which the mutations were found in female and male progeny respectively. The progeny which were known to carry X chromosomes derived from the treated male larvae were the sons from the crosses of treated males by yellow attached-X females and the daughters from the cross of treated males by treated females. These progeny totalled only 3546 altogether, and among them no cases of reverse mutation were found. The progeny which were known to carry treated X chromosomes derived from the female larvae *only* were the sons of the treated females. These numbered 10,011, and among these but one reverse mutation was found. The other 5 cases of reverse mutation all happened to occur in the daughters of treated females that may or may not have been mated with treated males—there being 7,773 such daughters in all. In these cases, then, the sex of the parent in which the mutation had occurred was uncertain.

In calculating the rate at which reverse mutations are produced from X-rayed larvae, we should count the case in which two mutations had occurred in the same culture as two distinct mutations, no matter whether or not they really had had a common origin, since the flies in which they

appeared would also have been counted separately, as non-mutants, if no mutations at all had appeared in them. The number of mutants being thus 6, what should our total number be, into which this is to be divided? The males, which constitute the total number of flies known to contain but one treated X, number 12,427. When we modify the numbers in the two last groups, rayed at D8, to make them equivalent to flies rayed at D5, the total number of males may be represented by the figure 13,980. The females in the third row from the last, which constitute all the flies known positively to contain two treated X's, number 1090. This latter number, then, should be doubled, to represent the number of X chromosomes here tested; it then becomes 2180. The remainder of the flies—all females of doubtful paternity—number 7,773 which, when modified to give the total number on the D-5 basis, becomes 8,969. If all these flies had been derived from treated fathers, the X's tested in them would be twice this number, or 17,938. Adding this number to those from the other two groups (representing progeny of treated males, and of both parents treated, respectively), we obtain 34,098 as the maximum number of X chromosomes tested. If, however, we take the smaller figure (8,969) for the females of the uncertain class, thus assuming none of them to have had treated fathers, our sum becomes 25,129, which would represent the minimum number of X-chromosomes tested.

Dividing 6 into the above two respective totals, we find that the observed frequency of reverse mutations from forked to non-forked was somewhere between a minimum of 1 in 5,683 and a maximum of 1 in 4,188. On the basis of a t-12 dose, which was that used in computing the mutations in the previous sections, we have the frequency lying between a minimum of 1 in 2,186 and a maximum of 1 in 1612. It will be seen that this frequency is of a magnitude rather similar to that of mutations from red to white in sperm cells and in larval somatic cells, and is also similar to that for mutations of the normal allelomorphs of forked and of scute, so far as our previous figures will allow these to be computed.

c. Reverse mutations induced in spermatozoa

In order to have a basis for the comparison of the production of reverse mutations from flies rayed in larval and in adult stages, 855 tinged forked males were treated when adults, some with the D5 and others with the D10 dose. They were then mated to yellow females having attached X-chromosomes. As table 10 shows, a total of 11,298 male offspring bearing their father's X was obtained. Of these, 3,198 were derived from sperm

given the D10 dose; if the latter number is doubled to put the total count all on the basis of D5, the revised total is found to be 14,496.

There were two flies which showed mutations in the characters being studied. One of these had the left half of the thorax forked and the right half non-forked (forked reverse mutation No. 8). Cases like this are frequently found among flies arising from treated mature germ cells (MULLER 1927, 1928a, b, c); they are called "fractionals." In explanation of them, it is assumed that the chromosome in the mature germ cell may be precociously split, and that the mutation has taken place in the gene of one of the two halves. A fly developing from such a germ cell will usually have one half of the body bearing the mutation. These flies often fail to breed true to the mutant type. They breed true to it only if their germ

TABLE 10

Reverse mutations from forked to non-forked, from irradiated adult males.

DOSE	NUMBER OF BOTTLES OF TESTS	NATURE OF TESTS OF TREATED FLIES	MALE OFFSPRING	REVERSE MUTATIONS
D5	176	2 tinged forked males \times 2 X-X females	5,563	1 male; No. 8
"	49	3 tinged forked males \times 3 X-X females	1,502	0
"	24	4 tinged forked males \times 2 X-X females	603	0
"	22	3 tinged forked males \times 1 X-X female	432	0
D10	97	2 tinged forked males \times 2 X-X females	3,198	1 male; No. 9
Totals	368	855 tested males	11,298	2 males

cells have received the mutated gene. It will be seen from the tests to be reported in the next section that in the present instance the germ cells had received the non-mutated gene. The determination of the locus of this mutation by genetic means was accordingly precluded.

The second mutation involving the forked character resulted in a fly (mutation No. 9) which was tinged non-forked throughout except for the left posterior scutellar bristle; the latter was forked. Breeding tests, not yet completed, indicate that in this case a very "weak" allelomorph of forked, very nearly resembling the normal type, has arisen. We may then term the case one of "partial reverse mutation." The significance of the induction of multiple allelomorphs has already been considered. The present case adds another bit of evidence to show that they really can be induced.

Assuming that the "fractional" mutation and the "weak" mutation both occurred at the locus of forked, and giving the fractional a value of

one-half in our count, we find that $1\frac{1}{2}$ partial or complete reverse mutations occurred in this experiment among 14,496 flies, on the basis of a D5 dosage. That is the same as 1 in 9,664 for a D5 dose, or 1 in 3,717 for a t12 dose. Considering the numbers involved, this frequency is not significantly different from the value, 1 in 1,612 to 1 in 2,186, for a t12 dose, obtained in the experiment on treated larvae, although it does suggest that mutations of this kind may be more difficult to obtain by treatment of spermatozoa than of immature larval germ cells. If we average the results from the two kinds of treatments together, we obtain a reverse mutation frequency, on the t12 basis, lying between 1 in 2000 and 1 in 2,500. This would make the frequency still more like that for other loci (treated in the adult male).

Though the reverse mutation frequency from treatment of the adult males cannot be said to be decisively lower than that from treated larvae, yet it certainly is safe to conclude from these results that the frequency in the adults cannot be much *higher* than it is in the larvae. This is a somewhat surprising fact, in view of the independent and unequivocal results of HARRIS (1929a, b) and of HANSON and HEYS (1929b), showing that at least five times as many lethal sex-linked mutations can be obtained by treatment of mature sperm as by treatment of the primordial germ cells of the adult male. It cannot yet be concluded from this distinct difference between these results and ours that the mutations from forked to non-forked are necessarily different in the mechanism of their production from the mutations more commonly dealt with. If true, this would be a fact of considerable importance. It is, however, at least as likely that the difference between the results from these particular mutations and the others lies in the greater viability or vigor of multiplication, in the immature gonad, of the cells bearing the reverse mutants, as compared with those bearing lethals.

It has been suggested by MULLER (see HARRIS 1929a) that one possible explanation of the infrequency of lethals from irradiated immature germ cells, as compared with those from treated spermatozoa, may be simply that a large proportion of the lethal-bearing immature cells die or fail to multiply at as high a rate as the non-mutated cells (that is, that a germ-cell selection occurs), whereas in the mature sperm the lethal changes would not be effective owing to the non-functioning of the genes in spermatozoa (MULLER and SETTLES 1926), and to the further fact that the chromosomes tend to be split in two halves, only one of which may contain the mutant gene. (In immature cells, the two halves would come to lie in different cells, through subsequent mitoses.) The fact that the pre-

sent case, dealing with "mutant" genes that do not lower vigor, shows no such difference between the frequencies observed after raying immature and mature germ cells as is found in the case of lethals and semi-lethals, tends to support the above explanation but the latter is still to be regarded as only provisional.

d. Tests of the loci of the reverse mutations

Though it has been tacitly assumed in the foregoing account that the non-forked individuals resulted from mutations at the locus of forked, it was essential to make perfectly sure of this point by means of breeding tests, since upon it depended the answer to our major question—whether or not mutations can be induced in both of two genetically opposite directions. The alternative to a real reverse mutation at the locus of forked was, it may be recalled, the origination of a dominant "suppressor" of forked, by a change at some other locus. Disproof of the latter idea and proof of the former would be equivalent to one another.

One way of testing whether or not a suppressor is present at a different locus from forked, and in addition to it, has already been described in connection with the tests of the reversion of forked accompanying the apparition of spectacted. In this method, the chromosome in question is crossed to one having normal genes, so far as the forked character is concerned. In the F_1 females, if a suppressor is present, with forked, in one of the chromosomes, crossing over between their two loci will occur in a certain percentage of cases, yielding chromosomes which contain forked but not the suppressor, and so forked offspring will appear. If the non-forked effect is on the contrary due to an allelomorph of forked itself, the F_1 females, being homozygous for non-forked, will give rise only to non-forked X-chromosomes. It is desirable, when making this test, to have some way of ascertaining the frequency of crossing over, since, if crossing over is for some reason prevented, females containing a suppressor accompanying forked would fail to yield the forked cross-overs, and the result would thus simulate that to be expected in the case of a true non-forked allelomorph. The danger of a considerable reduction of crossing over occurring is not negligible, since gene rearrangements having such effects have been found to be induced rather abundantly by X-rays (MULLER 1928b).

Mutants 2, 4, and 7 were tested by the above method. They, or flies inheriting their mutated X-chromosome, were crossed to flies which were normal in respect to the forked character but contained Bar eyes. The F_1 females, carrying in one X-chromosome tinged and the newly arisen

TABLE 11

Cross: $\frac{w^t F}{B} \text{♀} \times w^f \sigma^{\text{♂}}$ (where w^t = tinged, F = non-forked due to reversal, f = forked, and B = bar).

NON-CROSS OVERS					CROSSOVERS BETWEEN w^1 AND B				PERCENT OF CROSSOVERS
NO.	BAR FEMALES	TINGED FEMALES	BAR MALES	TINGED MALES	WILD TYPE FEMALES	TINGED BAR FEMALES	WILD TYPE MALES	TINGED BAR MALES	
2	139	127	135	139	106	111	122	121	46.0
4	126	81	172	156	99	105	131	129	46.4
7	142	144	118	123	126	117	114	116	47.3

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been a true reversal, and not due to the origination of a suppressor. Since the locus of Bar is only about a fifth of a unit from that of forked, the Bar character in this cross serves very accurately to inform us of the exact locus of the gene in question, while the tinged-forked crossovers serve to show whether the general frequency of crossing over throughout the chromosome is normal.

The counts, as before, were continued until 1000 flies had been obtained in each case. Since the heterozygous females had been backcrossed to triply recessive males (tinged forked non-Bar), the daughters as well as

TABLE 12

Tests for loci of reverse mutations No's. 3, 5, and 6. The counts are based on the first 1000 flies.

$$\text{Cross: } \frac{w^t F}{f B} \text{ } \varnothing \times w^t f \sigma^{\text{♂}} \text{ (symbols as in table 11).}$$

NON-CROSS OVERS					CROSSEVER BETWEEN w^t AND f					CROSSEVER BETWEEN FORKED AND BAR AS INDICATED
NO.	FORKED BAR FEMALES	TINGED BAR FEMALES	FORKED BAR MALES	TINGED MALES	WILD TYPE FEMALES	TINGED FORKED BAR FEMALES	WILD TYPE MALES	TINGED FORKED BAR MALES	PERCENT OF CROSS OVERS	
3	155	103	155	130	107	98	145	105	45.5	1 Bar 1 tinged forked
5	164	124	115	119	119	113	125	120	47.7	1 Bar
6	137	120	148	118	105	121	122	127	47.5	1 Bar 1 forked

the sons were again of value. It will be seen that in cases 3 and 6, respectively, there were just two crossovers between forked and Bar in the entire thousand, and in case 5, just one. This result agrees with surprising nicety with the standard percent of crossovers between Bar and the locus of forked—0.2 (STURTEVANT 1925). At the same time, the percent of crossovers between the loci of tinged and Bar is again quite normal (ranging from 45.5 for No. 3, through 47.5 for No. 6, to 47.7 for No. 5), so that there is no reason to suppose that the low percent of crossovers between forked and Bar in these cases is due to reduction of crossing over. These mutations also are accordingly to be classed as reversals at the locus of forked itself.

In order to complete the account of the genetic behavior of the mutations in this experiment, a record is presented in the appendix of the crosses which were made of each of the original mutant flies derived from treated larvae, and the counts of progeny obtained from these crosses. These

are not given here, as in all these cases the results were regular, showing the features to be expected, respectively, of females homozygous for tinged and heterozygous for forked, and of the male containing tinged non-forked.

The "fractional" male derived from the treated sperm, on the other hand, gave the results expected of an ordinary (tinged) forked. When crossed to a virgin forked female (of the red-eyed "849" forked stock), it produced 52 red-eyed and typically forked females and 52 red forked males. Although the locus of the mutation could not be determined here, in view of the mutant gene's having been received only by the somatic tissue, nevertheless, in view of the fact that all six of the preceding apparent reversals had really occurred at the forked locus, it is highly probable that here too this was the locus which had undergone the change.

The "partially reverse" mutant male, having tinged eyes and but one forked bristle, was also mated to a virgin 849 forked female. It produced 54 red males with typical forked bristles (their X being derived from their forked mother) and 57 red females which were very weakly forked, overlapping the normal type to some extent so that some appeared normal. Thus the mutation was proved to be sex-linked and partially dominant to the typical forked from which it arose. Further tests of it are being made to determine whether typical forked can be recovered from it by crossing over, but it is very likely, in view of the results with other "weakly forked" allelomorphs, that this mutation also was in the forked locus itself.

e. Radiation as the cause of the reverse mutations of forked

In order that definitive evidence might be obtained as to whether it was the radiation which was causing the above reverse mutations or some other peculiarities of the environment or the stock, control series of flies of the same stocks were carried along parallel to the experimental series and were handled in a similar manner, except that no X-ray treatment was given. This was especially important in the present instance, where reverse mutations were being looked for, since the general *Drosophila* work does not furnish nearly as extensive an indication of the frequency of reverse mutations, from the abnormal type back to the normal, as in the direction, normal to abnormal, owing to the fact that the vast majority of ordinary cultures contain flies which to begin with have the normal allelomorph of any particular gene. The counts from these control matings are all listed in table 13.

It was aimed to examine as many control flies as experimental, in order that the comparison might be adequate. As the totals show, 11,300 female

progeny were examined, representing 22,600 untreated X chromosomes, and 21,565 male progeny, making a total of 44,165 untreated X's altogether. Of these, it can readily be calculated from the table that 24,608 were derived from the mothers, and 19,557 were of paternal origin. In all the experimental series together, including those from treated larvae and from treated adults, there was a total of between 33,678 and 41,451 treated chromosomes (the exact number depending on how many of the females from treated larvae had been fertilized by their brothers). The number of untreated chromosomes subjected to the mutation test in the control series thus somewhat exceeded the number of treated chromosomes tested in the experimental series. Yet, in contrast with the 8 cases of reverse mutation found in the experimental series, not a single instance of this phenomenon was found in the controls.

TABLE 13

Controls for flies shown in tables 9 and 10. All from untreated flies.

NUMBER OF BOTTLES OF TESTS	NATURE OF TESTS	FEMALE OFFSPRING	MALE OFFSPRING	REVERSE MUTATIONS
31	1 tinged forked female ×2 tinged forked males	1,347	1,576	0
153	2 tinged forked females ×2 tinged forked males	6,467	7,823	0
46	1 tinged forked female ×1 tinged forked male	3,486	3,909	0
244	2 tinged forked males ×2 X-X females	..	7,224	0
39	1 tinged forked male ×2 X-X females	..	1,033	0
Total 513	1,324 tested flies	11,300	21,565	0

This result may be examined from the standpoint of the theory of probability. In doing this, we may for simplicity take the control and experimental lots as having been of exactly equal size, although in so doing we do not weigh the results quite as heavily in favor of a significant difference as would be justifiable. What, then, would be the chances, in two lots of equal magnitude, of finding all the exceptional events to occur in a given one of the lots, if there were really no determinate cause tending to produce the events in this lot rather than in the other one? As the chance for any one event to be in the given (that is, the treated) lot is $\frac{1}{2}$, and 7 at least of the events are independent, the chance for all 7 to be in this particular lot is $(\frac{1}{2})^7$, or 1 in 128. It can therefore be concluded with consider-

able confidence that this was not a chance result, and, since the X-ray treatment was the only factor consistently differentiating the numerous identically handled cultures of these two series, this must have been the agent which was responsible. The conclusions based on the present statistics are rendered even more secure when taken in connection (1) with the prior case of non-forked spectaclad arising after irradiation, and (2) with the mass of previous *Drosophila* work, in which, without treatment, no evidence of any such high incidence of reverse mutations had ever been noted, except in the special case of Bar crossovers and in DEMEREC's special cases in *D. virilis*.

X. MUTATIONS FROM INDUCED NON-FORKED TO FORKED

The induction of reverse mutations does not in itself provide proof that other changes than losses can be induced, unless it is accompanied by sound evidence of the induction of mutations in the opposite direction also (that is, from the normal to the abnormal type). That point mutations from the normal to some abnormal type, can, in general, be induced by radiation is now a well-established fact; hence the major emphasis of the present work fell upon proving the induction of the reverse mutations. Nevertheless, it was important to have specific evidence that at the locus here involved, that of forked, such changes (that is, to the abnormal form, forked), could be induced, for it was *a priori* conceivable that this locus, like that of Bar, might constitute a special case of some kind.

Considerable specific evidence of the induction of changes at this particular locus in the required direction, non-forked towards forked, had already been gained before the reverse mutation experiments with this locus were begun. This evidence has been briefly reviewed in an earlier section. Three independent heritable mutations from the original normal allelomorph towards forked, following X-ray treatment of the sperm, were there described. Two of them resulted in typical forked, and one in a "weakly forked" allelomorph. This evidence, taken in connection with the fact that only 9 sure mutations from normal to or towards forked had been noted in all the rest of the *Drosophila* work, at COLUMBIA and elsewhere (MORGAN, BRIDGES and STURTEVANT 1925), despite the fact that the normal allelomorph had been present to start with in the great majority of the cultures, often checked by other markers, made it extremely probable that the present mutations owed their origination to the X-ray treatment.

It was decided, however, to conduct some special experiments on this point, in order to determine whether this production of forked could be

repeated, and also in order to take advantage of the opportunity thus afforded to run an experimental series having parallel controls, handled, except for the irradiation, in exactly the same fashion. In conducting these tests, it was thought to be advantageous to introduce a peculiar modification which the previous occurrence of the reverse mutations had made possible. This special feature consisted in using both for the experimental series and the controls, not the original normal allelomorph of forked, but one of the non-forked genes which had arisen from forked by reverse mutation under the X-ray treatment. If, then, forked was found to arise (with a frequency beyond that expected or found in untreated material), the case for reversibility of the mutation reactions would be made even stronger than if the mutations had been induced in material that had not originally been derived from forked. For then a cycle (forked \rightleftharpoons non-forked) would have been completed, all under the influence of irradiation.

For these experiments on the reversibility of the "artificial" non-forked, flies were used from a homozygous stock of tinged non-forked that had been established from reverse mutation No. 3. In the first set of experiments, larval stages of these tinged non-forked flies were treated at 72-84 hours with the D5 dose.

The males derived from the treated larval stages were tested in two ways. Some were mated to yellow females with attached X's and others to $\delta 49$ forked females. In the former test, only the male offspring would be able to show mutations to forked derived from the father. In the latter test, by $\delta 49$ forked females, only the female offspring could reveal such mutations. In this latter cross, in addition to forked females due to mutation, there was also the possibility of forked females due to non-disjunction, and to non-virginitiy of the mother, that might in rare instances have carried sperm from the $\delta 49$ forked stock. The forked mutants would, in the breeding test, prove to be heterozygous for tinged, while the forked females of the other two classes would be homozygous for red. Females of the latter two classes could usually be distinguished from one another by whether or not they produced non-disjunctional offspring.

The test to the attached-X females yielded 9,738 male offspring. Among these, no mutation to forked was found (table 14, lines 1 to 3). In the test to $\delta 49$ forked females, 3034 female offspring were produced. Among these, there were six forked. Two of these forked females came from the same bottle, all the others from separate bottles. It was suspected that at least some of the six forked females were due to secondary non-disjunction, since eight exceptional males were found in the same series of bottles. Ac-

cordingly, the forked females were all mated to white forked males to see whether they were heterozygous for tinged, and whether exceptional males would be produced. The results showed that three of the females had been produced by secondary non-disjunction and two were non-mutants that were in all probability due to non-virginity, since no non-disjunctional offspring were produced. The remaining forked female proved to be a clear case of a mutation to forked at the locus of forked (forked mutation No. 4 in table 14), for, when crossed to white forked males, this female gave rise to 25 red forked and 25 light tinged forked daughters.

Three hundred and sixty-four virgin females derived from the treated larvae were also tested for mutations. This was done by crossing them to ♂49 forked males. In this cross, both female and male progeny would reveal mutations to forked. The tests yielded 9,339 females and 8,886 males. No mutation to forked was found among them (table 14).

TABLE 14
Mutations from non-forked to forked, from flies derived from irradiated larvae.

AGE OF TREATED LARVAE	DOSE	NUMBER OF BOTTLES OF TESTS	NATURE OF TESTS OF TREATED FLIES	REGULAR FEMALE OFFSPRING	MALE OFFSPRING	MUTATIONS
(hours)						
72-84	D5	113	2 tinged males × 2X-X females	..	4,227	0
"	"	134	3 tinged males × 2X-X females	..	4,304	0
"	"	48	4 tinged males × 2X-X females	..	1,207	0
"	"	67	2 tinged males × 2♂49 forked females	3,029	..	1 forked female; No. 4
"	"	182	2 tinged females × 2♂49 forked males	9,339	8,886	0
Totals		544	1,318 tested flies	12,368	18,624	1

In addition to the tests of flies treated in the larval stage, another set of experiments of similar magnitude was carried out on the same tinged forked stock, the difference in method being that in this case the treatment was applied to the adult flies. The treated males were mated to attached-X females, and the treated females to ♂49 forked males. As shown in table 15, there were 11,772 sons examined that were derived from the treated adult males. Three mutant forked males were found among these (forked mutations Nos. 5, 6 and 7).

Mutation No. 5 was tested by crossing it with a female containing the "C₁B" complex in one X chromosome and *s_c v f b_h* in the other. There were produced 30 Bar females, 33 forked females, and 33 males showing *s_c v f*.

The fact that the non-Bar females (and not the Bar females) showed the recessive forked character proved that the variant male carried forked as a germinal mutation, and it was also clear that this mutation must have been at the same locus as the familiar forked. Mutation No. 6 was clearly a fractional, since one-half of the thorax (the left half) was forked and the other half non-forked. In matings with yellow attached-X females it proved to be sterile. Mutation No. 7 was tested, like No. 5, by crosses with females containing the "C₁B" complex in one chromosome and *s_c v f b₁* in the other. The count of offspring showed 43 Bar females, 28 wild-type females, and 31 males showing the characters of *s_c v f*. Since the non-Bar daughters were not forked, it was highly probable that this mutation had been confined to the somatic tissue, that is, that it was a "fractional."

TABLE 15
Mutations from non-forked to forked, from irradiated adult flies.

DOSE	NUMBER OF BOTTLES OF TESTS	NATURE OF TESTS OF TREATED FLIES	FEMALE OFFSPRING	REGULAR MALE OFFSPRING	REVERSE MUTATIONS
D5	312	2 tinged males × 2 X-X females	..	9,552	3 tinged forked males; No's 5, 6 and 7.
"	45	3 tinged males × 3 X-X females		2,220	0
"	211	1 tinged female × 249 forked males	8,452	7,601	0
Totals	568	970 tested flies	8,452	19,373	3

The treated females in this series, mated to 849 forked males, gave 8,452 female and 7,601 male progeny. No mutations to forked were found among them.

All of the controls for the experimental series recorded in tables 14 and 15 are given in table 16. The total numbers counted in the experimental series dealing with treated larvae, in the experimental series dealing with adults, and in the controls, are all of similar magnitude. There was, in the controls, a total of 34,798 flies, including as many tested chromosomes, of which 14,450 were of paternal and 20,348 of maternal origin. There was one forked female among these flies, derived from a culture in which two tinged non-forked males had been mated by two 849 forked females. It was in the series from treated larvae carried on parallel to this that the two forked females produced by non-virginity had been found. Tests of the forked female here in question proved that she, too, had been produced in this way (by a previous mating of a 849 forked male

by a ♂49 forked female), since she bred as a homozygous red-eyed forked fly. There were no other forked flies among the controls. Hence, there were no mutations to forked in the control series.

The present data on the production of forked from non-forked are not extensive enough to be very informative from a quantitative standpoint concerning the frequency of these induced mutations; nevertheless, some comparisons may be of interest. If we total all the data from tables 14 and 15 together, we find that 58,813 progeny of treated parents were examined; these would be represented by 22,646 on the basis of a t12 dose. Counting the two fractionals as each equivalent to $\frac{1}{2}$, we have a total of 3 complete mutations to forked among these, or 1 in 7548 on the t12 basis. This is about 150 times the frequency calculated from the figures 9 in 10,000,000

TABLE 16
Controls for flies shown in tables 14 and 15. All from untreated material.

NUMBER OF BOTTLES OF TESTS	NATURE OF TESTS	FEMALE OFFSPRING	MALE OFFSPRING	REVERSE MUTATIONS
266	2 tinged males × 2 X-X females	..	12,121	0
24	2 tinged males × 2♂49 forked females	2,329	..	0
46	1 tinged female × 2♂49 forked males	2,179	2,225	0
190	2 tinged females × 2♂49 forked males	7,911	8,033	0
526	1,206 tested flies	12,419	22,379	0

which roughly represents the results from previous *Drosophila* work on untreated material. It is considerably lower than the frequency, 1 in 2,000 to 2,500 at the t12 dose, for the lumped data on forked to non-forked mutations, but it is also lower than the frequency (1 in 3120) found for non-forked to forked mutations in the sum of all previous X-ray work. If we add together the present and the previous non-forked to forked data, we find an equivalent of 6 "complete" mutations to forked in 32,010 on the t12 basis, or 1 in 5,335, which is about 200 times the apparent "natural" frequency. This is still only half the frequency found for the mutations in the opposite direction; yet the difference is of doubtful significance, particularly since it is quite likely that the lumping of the data from both sexes and from two different stages of the life cycle is illegitimate.

The fact that all but one of the 4 (or "3") mutants to forked in the more recent experiments were derived from treated sperm, although only a fifth of the progeny of treated flies were of such origin (the rest being from treated larvae), also merits some attention, for the opposite tendency appeared

to be at work in the mutations from forked to non-forked. While the figures on this point are not yet decisive, it is to be noted that this is the sort of result to be expected on the hypothesis of a "germ-cell selection," if the cells with the non-forked allelomorph be considered as having a tendency to multiply more quickly than those with the forked allelomorph. For, in that case, when forked larvae containing immature germ cells were irradiated, the non-forked mutant cells should be represented in the adult gonads in relatively greater abundance than that in which they actually originated, but when non-forked larvae were irradiated, the forked mutant cells should be present in the adult in relatively lesser abundance than that in which they originated, due to the higher rate of multiplication of their non-forked neighbor cells. In the adult sperm there would be no opportunity for such selective action.

The major interest of the present data lies, however, not so much in the exact mutation frequencies shown, but rather in the fact that mutations to forked occurred at all. The presence of two clear germinal mutations, and two somatic fractionals, among the treated series, and none among the controls, when added to the previously obtained data indicating the induction of forked, furnishes convincing evidence that irradiation can cause mutation in this direction. Taken by themselves, the data also make it probable that irradiation can cause a reversion of the very allelomorph which was itself so produced. Since it has been shown in the foregoing that mutation from forked to non-forked also can be induced, the links in our chain of evidence against the hypothesis of induced mutation being exclusively by loss have now been forged.

XI. VIABILITY OF THE INDUCED REVERSE MUTATIONS

An interesting point in connection with the question of the possibly destructive action of X-rays is whether the flies showing the induced reverse mutation are as viable as the non-mutated stock from which they arose. To test this point, tinged non-forked males from each stock of four of the reverse mutations (Nos. 3, 5, 6, and 7) were backcrossed to tinged forked females of the original stock. The F_1 flies were inbred, four pairs to the bottle, and the F_2 males examined and counted. Obviously, if the flies bearing the reversed genes of these four stocks are as viable as those bearing the original forked gene, then the two types of males (tinged forked and tinged non-forked) expected in the F_2 generation, should appear in equal numbers.

The results obtained in this test are recorded in table 17. The F_1 flies were kept in the culture bottles for six days and then removed. The

counts of the F_2 males were made daily for six successive days after they began emerging. This was done in order to determine whether there was any difference in the time of emergence of the two types of males. An examination of the table shows that the tinged non-forked males emerged in greater numbers at first then did the tinged forked males. This is made clear if we take the counts in two-day periods. The number of non-forked males of the first and second counts is 325 as against 240 forked males. Thus more than fifty-seven percent of all of the males of this period belong to the reverse mutation type. In the third and the fourth counts, there are 467 non-forked males and 521 forked males. About forty-seven percent of all of the males in these two counts were non-forked. Finally, in the fifth and sixth counts, there are 291 non-forked and 309 forked males. These counts show clearly that there is a strong tendency for the reverse mutation males to emerge earlier than the forked males. In this sense, they may be said to be more vigorous. This fact is of particular interest when considered in connection with the possibility previously broached, that the non-forked cells may multiply faster than the forked cells in the gonad, causing a "germ-cell selection."

TABLE 17

Showing results of tests for viability of four of the reverse non-forked mutations.

DAILY COUNTS	NO. 3		NO. 5		NO. 6		NO. 7		TOTALS	
	TINGED MALES	TINGED FORKED MALES	TINGED MALES	TINGED FORKED MALES	TINGED MALES	TINGED FORKED MALES	TINGED MALES	TINGED FORKED MALES	TINGED MALES	TINGED FORKED MALES
1st	100	69	7	4	0	1	73	62	180	136
2nd	55	27	27	25	27	28	36	24	145	104
3rd	68	67	79	90	54	79	65	44	266	280
4th	77	107	30	53	26	18	68	63	201	241
5th	41	68	18	19	19	19	53	49	131	155
6th	20	26	48	31	48	47	44	50	160	154
Total	361	364	209	222	174	192	339	292	1,083	1,070

It is, however, to be noted that there is no significant difference in viability in the two types of males; when the counts from all periods are added together, it is found that there were 1083 non-forked and 1070 forked males in all. When we consider that either of these allelomorphs can be produced by X-rays from the other, we see that these tests lend no support to the view that the gene change produced by X-rays is necessarily of an injurious nature, even though it is to be expected that injurious

changes would, as a matter of chance, happen more often than beneficial ones, all loci considered (MULLER 1923).

XII. REVERSE MUTATIONS IN EYE COLOR

Throughout the examinations of all the flies in the experiments with forked, a careful search was made for possible reversions in eye color, from tinged to red or to some of the intermediate allelomorphs of white. It may be recalled that this was part of the original purpose of the experiments. As was reported in section IV, previous experience in trying to produce somatic reverse mutations in eye color had indicated that if such mutations could be induced by X-rays, they would occur at very rare intervals. In all the previous radiation work on larval or somatic mutations in which white or some other mutant allelomorph of white was used,—work involving altogether 4661 treated flies in which it would have been possible to detect reverse mutations at this locus,—only one certain case of a mutation to a more deeply pigmented condition had been found. This was a male apricot fly that had been treated in the mid-larval stage. The mutant area was composed of twenty-nine ommatidia which were distinctly darker than apricot, but slightly lighter than red (PATTERSON 1929b).

In the experiments on tinged forked, none of the flies showed areas or individual ommatidia of darker color. In these experiments there were however three males, two from one culture and one from another, that had red eyes and were non-forked. These came out of cultures in which the males had been treated during the larval stage and crossed to yellow attached-X females as a test for reverse mutations. The attached-X females came from a stock in which the males had the $\delta 49$ (non-forked) composition. It was therefore suspected that the three males might have come from females that had been fertilized before they were collected. Accordingly, each red-eyed male was mated to females containing one chromosome with the “C₁B” complex and one with the genes $s_e v f b_b$. The F₁ females containing the $s_e v f b_b$ chromosome were then tested for crossovers. In each of the three cases, practically no crossovers were found among the F₂ males; this showed that these males were not the result of reverse mutations, but were in reality $\delta 49$ males.

These results, combined, leave no doubt that reversions of tinged or white towards red cannot be induced with nearly the same frequency as reversions of forked towards non-forked, although mutations from red towards white can be induced about as readily as from non-forked towards forked. Similar conclusions have been arrived at independently by TIMOFEEFF-RESSOVSKY (1929b, 1930), in work to be referred to later.

Hence the mutations at different loci, as well as the different possible mutations at the same locus, have differing frequencies depending on rules of their own which doubtless are a reflection of their chemical composition.

XIII. THE TWO SCUTE MUTATIONS

All the male offspring of the treated tinged and tinged forked flies which carried an X-chromosome from their treated parent would be capable of showing scute if this mutation had occurred. As there was a particular interest attaching to this locus on account of the reverse mutations which had previously been found there, all such male offspring were carefully examined for this character. A summary of the tables shows that the following total numbers of male offspring were produced: 24,067 (27,265 at D5) carrying X's derived from treated adult males; 7,601 (all at D5) carrying X's from treated adult females; 12,154 (12,253 at D5) carrying X's from treated larval males; 18,897 (19,768½ at D5) carrying X's from treated larval females. The "grand total" is 62,719 (66,887½ at D5). Among these two scute flies were found. There were none in the 43,946 control males.

One of the scute males was a tinged non-forked, derived from a tinged non-forked male treated as an adult with the D5 dose, crossed by a yellow attached-X female. The other was a tinged forked derived from the similar cross of flies of this composition. It is worth noting that both were derived from treated mature spermatozoa, although the males of such origin did not form half of the total count. Both males were tested for germinal scute by mating them to virgin females homozygous for scute, carrying in one X chromosome the "C₁B" complex (which contains scute) and in the other X the genes *s_c* *v f b₁*. The offspring of the scute tinged non-forked male consisted of 8 scute females, 12 scute Bar females, and 14 scute vermilion forked males. The offspring of the scute tinged forked male consisted of 30 scute forked females, 22 scute Bar females, and 25 scute vermilion forked males. The presence of the scute in all the daughters in both instances proved that the mutation was germinal, and that it was allelomorphous to (that is, in the same locus as) the gene for the familiar scute character. Observation showed that in both instances all the bristles were affected in the typical manner.

XIV. DISCUSSION OF RELATED WORK, AND GENERAL CONSIDERATIONS

The demonstration that mutations at the locus of forked can be repeatedly obtained in both of two opposite directions in X-rayed and not in control populations of comparable magnitude affords convincing evi-

dence that high-frequency irradiation is capable of causing changes in the genes which are not of the nature of losses. Verification is thus provided of the inferences based on the less extensive experiments with the scute locus, and on the less direct evidence from other sources (nature of the physical action of radiation, partial separating of the induction of chromosome abnormalities from that of point mutations, apparition of the dominant eyeless not resembling the chromatin loss of the same region, induction of multiple allelomorphism).

The extensive work of TIMOFEEFF-RESSOVSKY (1929 a, b, 1930) in irradiating mutant stocks of *Drosophila*, which was carried out independently and came to our attention while our more recent experiments on reverse mutations were already under way, was undertaken with the same primary object and has given parallel evidence of the occurrence of reverse mutations. In his earlier experiments TIMOFEEFF-RESSOVSKY irradiated eggs and larvae of white and of eosin eyed flies, with the object of obtaining facet changes in opposite directions. Among 2986 flies from treated larvae having the gene for white eye one case of a group of 3 red facets was found, and one case of a single facet of intermediate color, while among 1407 flies from treated larvae of eosin stock, a case of a group of 3 red facets was found. Thus it was proved that mutations at this locus could be induced but it also became evident (as in our work) that mutations from white towards red must be much less frequent than those of red towards white.

In his later experiments TIMOFEEFF-RESSOVSKY irradiated adult males of white and of various multiple recessive stocks, namely, *s_c w^e e_c*, *y c_v v f*, and *Xpl, IIIpl*, and "rucuca" (*r_u h t_h s_i p s_r e^e c_a*). In all, 13 reversals to the normal character were found, distributed as follows: from scute (3 times), esin, echinus, crossveinless, vermilion, forked (twice), hairy, pink (twice) and sooty. In the case of all except the echinus, vermilion and sooty reversals it was found possible to breed the flies and establish the fact that the change was genetic, and the presence of the other characters as "markers" served to show that no contamination had occurred. At a number of the loci involved—those of scute, white, forked and pink,—one or more mutations in the direction, normal to abnormal, have also been observed by TIMOFEEFF-RESSOVSKY after raying. He has in his discussion emphasized the adverse significance of these results for the loss hypothesis.

It will be seen that TIMOFEEFF-RESSOVSKY's experiments on reverse mutations and ours are mutually confirmatory and complementary. His were carried out in such a way that reversals in a considerable number of loci could be found, ours in such a way that a considerable number of

mutations in opposite directions could be demonstrated to occur at a given locus, in both cases similar total numbers of mutations having been obtained.

In view of his results and ours combined there can be no doubt that the conclusions which we on the basis of our own work have reached in regard to the locus of forked and, secondarily, of scute can be generalized so as to apply to a large proportion of the existing loci.

Persistent adherents of the idea of mutation by loss would now be forced back into some very specialized modification of this hypothesis, such as that all mutations are merely quantitative—losses *and* gains of genetic material, of a kind previously present in the nucleus,—as postulated for instance by GOLDSCHMIDT (1927, 1928), but the burden of proof in regard to any such contention would now be clearly theirs. It is difficult to conceive a simple mechanism which would be able to actually increase the amount (or permanently increase the activity) of gene material of any kind that happened to be “struck.” Such increase, on this hypothesis, would involve the transformation of neighboring non-genic material into genic material in a very particular way, specific for each case, and yet it would have to be supposed that the active agent was unable to cause any qualitative change whatever in the genic material that was already there. Addition of material at the given locus through some process resembling translocation, that is, a displacement, would be virtually ruled out by reason of the facts (1) that the supposed “addition” is usually found to be of a particular kind, for a given locus, (2) that there is never evidence of a complementary loss occurring in these cases, (3) nor of a complementary locus in which the reverse of these changes may, at other times, be found. Finally, there is the empirical evidence from the cases of multiple allelomorphism which have arisen following irradiation, showing that some of the different changes in a given locus show by no means purely quantitative relationships to one another in their phaenotypic expressions. From a theoretical standpoint, any “quantitative” hypothesis lacks what advantages the sheer “loss” hypothesis might have had, yet suffers from all the disadvantages of the latter and considerably more besides.

Since it is thus probable that irradiation can cause “random” changes of varied sorts in the inner composition of the genes, it becomes arbitrary to attempt to limit the kinds of changes which can be produced. The kind of change produced no doubt depends on the nature of the gene to begin with, the precise manner in which it was struck, and the arrangement, etc., of its own and surrounding atoms at the time. As in GALTON’s polygon

of variations, there would be certain more probable kinds of changes, but varied possibilities in all. Moreover, after one kind of change had occurred, not only reverse mutations but still different changes would theoretically be possible, which might in time accrue so as to result in a gene having a very different function from that which it had to begin with. In this manner, through the possibility of an *indefinite succession* of mutations in each locus, the way for continued evolution would be opened up.

At the same time, it should be borne in mind that many if not most of the characteristics of organisms have probably been developed to an optimal (and in some cases to a maximal) stage already, so that any further change would be likely to be somehow detrimental, disorganizing and often, in effect, reactionary. When some radical alteration takes place in the mode of living of the organism, through a peculiar change in outer conditions, or in some other character from the one primarily in question, then, perhaps, the optimal value for our given character becomes different and mutations previously detrimental are, in their new settings, progressive. Lacking such rare circumstances, really "progressive" changes might be of almost fabulous infrequency in organisms that had long become adjusted to their present mode of life. This would not mean, however, that changes chemically of the same general character as the "progressive" changes of the past were not still taking place nor that, at some time in the future when the outer or inner adjustments somehow become upset, such changes might not again have opportunity to cause a further advance in the organization of the race.

It might, to be sure, be a very difficult matter to study the further mutations possible in a given locus, following upon the initial steps with which we ordinarily deal in our mutation studies, since the familiar mode of phenotypic expression of mutations at that locus might well fail to govern the further changes, and the investigator would then have little clue as to what variations to look for and to test (supposing they were visible to his superficial observation at all). Nevertheless, such studies may be undertaken eventually. The present work has corroborated the claim previously made that the X-ray treatment raises the frequency of mutations sufficiently to make practicable the study of changes in a particular locus, at any rate if attention is confined to certain types of changes at that locus. The work on the forked locus demonstrates this point. More work must be done before we can know whether the study of a succession of different changes in a given locus is practicable.

The above is one way in which the study of mutations produced by

radiation may be expected to have relation to the study of evolution. For, if our interpretation of the mechanism of production of the mutations based on our findings is correct, the varied and cumulative possibilities of the X-ray mutations would be like those existing for the natural mutations. The finding that the X-ray mutations are not confined to losses makes this conclusion considerably more probable. And the conclusion would also follow that in using the X-ray mutations in attempts at artificial evolution, we are employing an agent capable of causing an indefinite succession of changes, with as much chance of "progression" as there is in the evolutionary processes of nature.

SUMMARY

1. A consideration of the mechanism whereby X and related rays affect matter gives ground for concluding that changes of varied kinds in the composition of the genes are produced by such radiation.

2. Data are presented which show that in mature spermatozoa X-rays produce a much larger proportion of breakages and reattachments of portions of the chromatin ("displacements") in comparison with a given number of "point mutations" than they produce in immature germ cells (of females). This indicates that the "point mutations" are not simply breakages and reattachments on a small scale.

3. Other facts concerning the manner of origination of the induced "point mutations," the phenotypic effects of those at different loci, and their relations of allelomorphism and dominance, lead to the same conclusion.

4. The origination of multiple allelomorphism at three different loci, following irradiation, is described. At the locus of scute, two mutant allelomorphs—scute and scutex—were found; at that of forked, three—forked, a "weakly forked," and a "very weakly forked" allelomorph, the latter derived from forked; at that of white, three, or more probably, four—(1) white, (2) an allelomorph resembling ivory and derived from eosin, (3) apricot, and (4) a probable allelomorph darker than apricot and derived from it.

5. The comparatively small total numbers among which these cases were found, as contrasted with the great rarity of similar cases in previous work with untreated material, make it highly probable that the irradiation was the causative agent in their production.

6. These multiple allelomorphs furnish the customary combination of evidences against the hypothesis of "presence and absence" as applied

to the mutations produced by X-rays, which has in the past served to undermine the same hypothesis when applied to mutations in general.

7. An account is given of the origination and characteristics of a dominant gene for reduced eyes ("Dominant eyeless") located in the fourth chromosome and probably allelomorph to the known recessive "eyeless."

8. The fact that, when one fourth chromosome is absent and the other is present and normal (the "haplo-IV" condition), no such effect is produced as when one fourth chromosome contains this mutant gene and the other is normal (the heterozygous Dominant eyeless condition), indicates that this mutation did not consist of a loss of genetic material. Since the presence of an additional fourth chromosome (the "triplo-IV" condition) also fails to give the "eyeless" effect, the mutation does not seem to have involved the increase of a gene in the fourth chromosome. And the fact that allelomorph eyeless changes have occurred at this locus on a number of occasions gives evidence that the change did not consist of an addition to the fourth chromosome of some genetic material from a non-homologous chromosome. It is, therefore, likely that what occurred was a change in the composition of a gene.

9. Experiments are described in which attempts were made to produce reverse mutations, to or towards red eye, in the gene for white and in other mutant allelomorphs of the white locus. Only one case of such a change was found (apricot to a darker color, lighter than red) although there would have been numerous cases in all the material examined if mutation towards red had been inducible with as high a frequency as the change in the opposite direction, or with as high a frequency as the reverse mutations at the locus of forked (to be referred to).

10. Scute or some other mutant allelomorph of this locus has arisen three times from the normal allelomorph in our X-ray work. On the other hand, among 208 X-chromosomes derived from X-rayed females, the mutant allelomorph scute reverted to non-scutel on two independent occasions. Hence it is very likely that mutations can be induced in both of two opposite directions at this locus.

11. An experiment is described in which, following treatment of male larvae, a reverse mutation from forked to non-forked (at the locus of forked) occurred simultaneously with another visible point mutation, from normal to spectacled eye (at the locus of lozenge), in the same chromosome.

12. In order to determine definitely whether reverse mutations, from forked to non-forked, could be produced by irradiation, experiments were carried on on an extensive scale with flies carrying tinged eyes and forked

bristles. Both larval and adult stages, and both sexes, were rayed, in different divisions of the experiment, and an approximately equal number of non-treated flies were bred in the same way, as controls. In all, 8 cases of mutation to or towards non-forked (7 being of certainly independent origin) were observed among progeny of treated flies, and none among progeny of controls. In 7 of the cases, the mutation involved the germ tract of the progeny in which it was found, and in the other case (derived from treated sperm) the mutation involved part of the soma only, being of a "fractional" nature.

13. Tests of the 7 heritable mutations showed all of these to be "point mutations" that had occurred at the locus of forked; they were therefore true reverse mutations of the forked gene.

14. The chance of obtaining all 7 independent reverse mutations in the treated and none in the control group would have been only 1 in 128 if the irradiation had not been effective in producing these mutations.

15. Similar experiments were then undertaken with stock of tinged non-forked that had been derived by reverse mutation from forked, to determine whether this could be caused to mutate again to forked. Two inheritable mutations to forked and two "fractionals" confined to the somatic tissue of the flies showing them (the latter derived in both cases from treated spermatozoa) were observed in a count of 58,817 progeny of treated flies. None were found in 34,798 controls. In previous irradiation experiments three other mutations to or towards forked had been detected among 12,482 progeny of treated males, and none in over 10,000 controls. The results suggested, though they did not prove, that mutations from non-forked to forked may be more readily obtained by treatment of the adult males than of the larvae, and that mutations from forked to non-forked may be more readily obtained by treatment of larvae than of adult males. Both these results would be expected if there is a "germ cell selection" among primordial and gonial germ cells, by reason of a faster multiplication rate of non-forked than of forked-bearing cells.

17. Tests of the above two heritable mutations from the later experiment, and of the three other mutations to or towards forked that had been found previously in the progeny of irradiated flies, showed all of these to be allelomorphic to the known forked.

18. The occurrence of these five heritable and two fractional (probable) mutations to forked in radiated material, when considered in connection with the extreme rarity of such mutations in non-radiated material, furnishes convincing evidence that the irradiation was the cause of the mutations in this direction also.

19. The above demonstration that mutations can be produced by irradiation in both of two opposite directions at the same locus, and that, in fact, a cycle of mutational change can be completed, is irreconcilable with the view that all mutational changes by X-rays consist of losses.

20. There are also grave objections cited in the text against interpreting these results as involving mere increases and decreases in the amount of genic material at the locus in question, or as involving displacements of portions of the chromatin. The non-quantitative relationships shown by the multiple allelomorphs produced after irradiation at the locus of white and possibly at that of scute bear witness to the same conclusion.

21. Comparisons of the viability of flies carrying forked with those carrying non-forked that were produced from this forked showed no significant difference in this respect. There was, however, a noticeably higher speed of development on the part of the non-forked flies. In this sense, the X-ray mutation to non-forked had caused an increase in vigor.

22. These results in general lead to the same conception as arrived at by consideration of the nature of the physical action of radiation, namely, that the induced point mutations are changes in the chemical composition of the genes, that they may be of varied kinds, and that they probably are, through the possibility of the accumulation of such changes, endless in their eventual potentialities. In other words, so-called "progressive" mutations can probably be produced by artificial irradiation in cases where there is the possibility of their occurring at all.

APPENDIX

Results of crosses of apparent reverse mutants derived from treated larvae.

Reverse mutation No. 2

This tinged female fly was mated to a forked Bar male. She gave 17 forked Bar females, 11 Bar females, 9 tinged forked females, 3 tinged females, 17 tinged forked males, and 21 tinged males. These results show that she was heterozygous for forked, and that she had been fertilized by a tinged forked male before the cross to the forked Bar male was made. Tinged (non-forked) males from the above count were crossed to virgin Bar females from stock of Bar, and yielded 31 Bar females and 32 Bar males. The resulting Bar females were then backcrossed to tinged forked males from stock, yielding the count shown in the top row of table 11.

Reverse mutation No. 3

This tinged female was mated to scute garnet forked outstretched males. She gave 20 tinged females, 10 tinged forked females, 25 tinged

males, 24 tinged forked males, 9 wild type females, and 4 forked females. These results show that she was heterozygous for forked and had been fertilized by her brothers. Tinged males (non-forked) from this culture were then crossed to virgin forked Bar females and yielded a count of 49 Bar females and 56 forked Bar males. The above Bar females were then backcrossed to tinged forked males, yielding the count shown in the top row of table 12.

Reverse mutation No. 4

This tinged female was mated to a forked Bar male, and gave 29 Bar females, 30 forked Bar females, 24 tinged males, and 18 tinged forked males. She was therefore heterozygous for forked, and virgin at the time of mating. Tinged males from the above count were then crossed to virgin Bar females from stock of Bar, and yielded 24 Bar females and 20 Bar males. These Bar females were then backcrossed to tinged forked males, yielding the count shown in the second row of table 11.

Reverse mutation No. 5

This tinged female was crossed to forked Bar males, and gave 31 Bar females, 32 forked Bar females, 11 tinged females, 6 tinged forked females, 27 tinged males, and 34 tinged forked males. She had been fertilized by her brothers, and was heterozygous for forked. The F_1 females, heterozygous for tinged, Bar, and forked, were backcrossed to tinged forked males, and gave the results shown in table 12, row 2.

Reverse mutation No. 6

This tinged female was mated to forked Bar males, and gave 28 Bar females, 28 forked Bar females, 28 tinged males, and 24 tinged forked males. She was therefore virgin at the time of mating, and was heterozygous for forked. The same test for crossovers was made as for No. 5 (see table 12, bottom row).

Reverse Mutation No. 7

This tinged male was mated to Bar females, and gave 82 Bar females and 98 Bar males. Females from this stock, heterozygous for tinged and Bar, were backcrossed to tinged forked males, giving the results shown in table 11, bottom row.

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